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Year: 2013

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## Chirality-Driven Folding of Short $\alpha$ -Lactam Pseudopeptides

Aizpurua, Jesus M ; Palomo, Claudio ; Balentová, Eva ; Jimenez, Azucena ; Andreieff, Elena ;  
Sagartzazu-Aizpurua, Maialen ; Miranda, José Ignacio ; Linden, Anthony

**Abstract:** Novel enantiopure pseudopeptide models containing a central  $\alpha$ -( $\alpha$ -lactam)-(Aa)- scaffold characterized by the combined presence of an  $\alpha$ -alkyl-  $\alpha$ -amino-  $\alpha$ -lactam (i+1) residue and a  $\alpha$ -substituted (i + 2) amino acid have been readily synthesized from  $\alpha$ -alkyl serines. The conformational analysis of such  $\alpha$ -lactam pseudopeptides conducted in CDCl<sub>3</sub> and DMSO-d<sub>6</sub> solutions using 1D- and 2D-NMR techniques revealed an equilibrium between  $\alpha$ -II turn and  $\alpha$ -turn conformers, which was ultimately modulated by the relative configuration of the  $\alpha$ -( $\alpha$ -lactam)-(Aa)- residues. Long-range chiral effects on the  $\alpha$ -lactam pseudopeptide conformers were also found when two (i) and (i + 3) chiral residues were attached to the termini of a central  $\alpha$ -( $\alpha$ -lactam)-(Aib)- segment. In such mimetics, heterochiral (i) and (i + 3) residues reinforced a  $\alpha$ -II turn conformer, whereas homochiral corner residues stabilized an overlapped  $\alpha$ -II/  $\alpha$ -I double turn motif. No  $\alpha$ -hairpin nucleation was observed in any instance. In good agreement with the conformers found in solution,  $\alpha$ -turned and open structures were also characterized by X-ray crystallography. Relative stabilities of the different conformers were estimated computationally at a B3LYP/6-31++G\*\* calculation level, and finally, a conformation equilibrium model based on steric inter-residual interactions around the  $\alpha$ -( $\alpha$ -lactam)-(i + 2)- segment was proposed to account for the observed chiral effects.

DOI: <https://doi.org/10.1021/jo302368y>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-82082>

Journal Article

Accepted Version

Originally published at:

Aizpurua, Jesus M; Palomo, Claudio; Balentová, Eva; Jimenez, Azucena; Andreieff, Elena; Sagartzazu-Aizpurua, Maialen; Miranda, José Ignacio; Linden, Anthony (2013). Chirality-Driven Folding of Short  $\alpha$ -Lactam Pseudopeptides. *Journal of Organic Chemistry*, 78(2):224-237.

DOI: <https://doi.org/10.1021/jo302368y>

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Journal:	<i>The Journal of Organic Chemistry</i>
Manuscript ID:	jo-2012-02368y
Manuscript Type:	Article
Date Submitted by the Author:	28-Oct-2012
Complete List of Authors:	Aizpurua, Jesus; Universidad del País Vasco, Departamento de Química Orgánica-I Palomo, Claudio; Universidad del País Vasco, Departamento de Química Orgánica I Balentová, Eva; Universidad del País Vasco, Departamento de Química Orgánica-I Jimenez, Azucena; Universidad del País Vasco, Departamento de Química Orgánica-I Andreieff, Elena; Universidad del País Vasco, Departamento de Química Orgánica-I Sagartzazu-Aizpurua, Maialen; Universidad del País Vasco, Departamento de Química Orgánica-I Miranda, Jose; University of Basque Country, NMR Service Linden, Anthony; University of Zurich, Institute of Organic Chemistry

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# Chirality-Driven Folding of Short $\beta$ -Lactam Pseudopeptides

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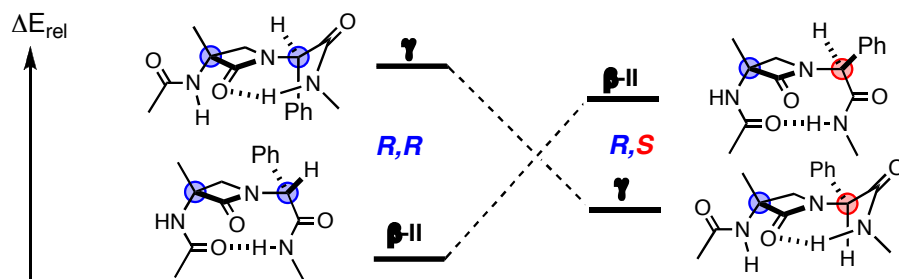
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ABSTRACT. Novel enantiopure pseudopeptide models containing a central  $-(\beta\text{-lactam})-(\text{Aa})-$  scaffold characterized by the combined presence of an  $\alpha$ -alkyl- $\alpha$ -amino- $\beta$ -lactam ( $i+1$ ) residue and a  $\alpha$ -

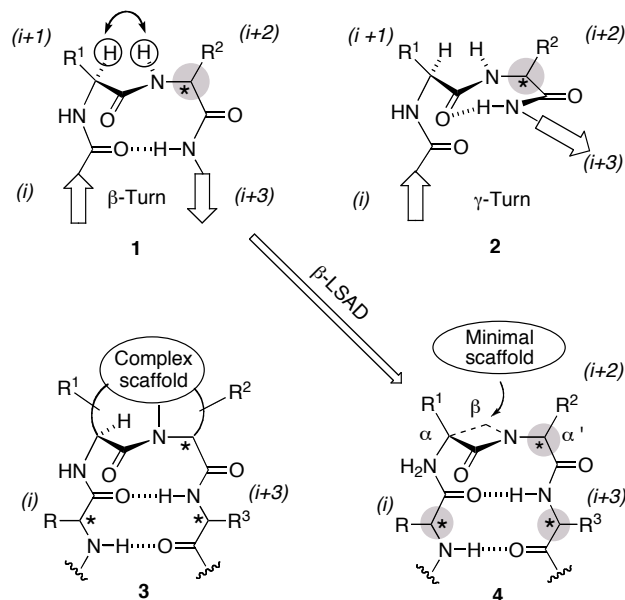
substituted ( $i+2$ ) amino acid have been readily synthesized from  $\alpha$ -alkyl serines. The conformational analysis of such  $\beta$ -lactam pseudopeptides conducted in  $\text{CDCl}_3$  and  $\text{DMSO}_{d6}$  solutions using 1D and 2D-NMR techniques revealed an equilibrium between  $\beta$ -II turn and  $\gamma$ -turn conformers, which was ultimately modulated by the relative configuration of the  $-(\beta\text{-lactam})\text{-(Aa)-}$  residues. Long range chiral effects on the  $\alpha$ -lactam pseudopeptide conformers were also found when two ( $i$ ) and ( $i+3$ ) chiral residues were attached to the termini of a central  $-(\beta\text{-lactam})\text{-(Aib)-}$  segment. In such mimetics, heterochiral ( $i$ ) and ( $i+3$ ) residues reinforced a  $\beta$ -II turn conformer, whereas homochiral corner residues stabilized an overlapped  $\beta$ -II/ $\beta$ -I double turn motif. No  $\beta$ -hairpin nucleation was observed in any instance. In good agreement with the conformers found in solution,  $\beta$ -turned and open structures were also characterized by X-ray crystallography. Relative stabilities of the different conformers were estimated computationally at a B3LYP/6-31++G\*\* calculation level and, finally, a conformation equilibrium model based on steric inter-residual interactions around the  $-(\beta\text{-lactam})\text{-(}i+2\text{)-}$  segment was proposed to account for the observed chiral effects.

**KEYWORDS.** Reverse-turn mimics;  $\beta$ -Lactams;  $\beta$ -Turns;  $\gamma$ -Turns; NMR spectroscopy.

## Introduction

Chirality is at the origin of the regular folding patterns occurring in proteins and natural peptides. It is well known that short “all-L” peptides may experience dramatic changes in their backbone conformation due to the replacement of a single residue by an achiral unit or a D-configuration amino acid.<sup>1</sup> This phenomenon was first found in natural peptides and is now widely used as a strategy to design peptidomimetics stabilizing reverse turn motifs such as  $\beta$ -turns<sup>2</sup> **1** and  $\gamma$ -turns<sup>3</sup> **2** (Figure 1, top). Indeed, changing the configuration of some backbone residues in functional short peptides may result in increased potency, altered selectivity<sup>4</sup> or improved proteolytic stability. Furthermore, these effects can become amplified when conformationally constrained lactam scaffolds **3** incorporating interresidual

bridges within contiguous C $\alpha$ H and NH positions (Freidinger lactams)<sup>5</sup> are embedded in the native peptide backbone (Figure 1, bottom). Formation of more complex structures, such as the doubly hydrogen-bonded  $\beta$ -hairpins **3** (antiparallel  $\beta$ -sheet nucleators) is also strongly dependent on the configurations of the terminal residues ( $i$ ) and ( $i+3$ ).<sup>6</sup>



**Figure 1.** Top: peptides folded into  $\beta$ -turn (**1**) and  $\gamma$ -turn (**2**) motifs. Bottom: Freidinger's lactam peptidomimetics (**3**), and  $\beta$ -LSAD concept to design minimally scaffolded  $\beta$ -lactam pseudopeptides **4**, differing from the parent peptides in a single carbon atom.

Unfortunately, most of the ( $i+1$ )-(i+2) dipeptide lactam surrogates used routinely as turn mimetics<sup>7</sup> possess comparatively large external fragments and their restraint elements (cycles) and recognition groups (R<sup>1</sup> and R<sup>2</sup>) are often crammed in the scaffold.<sup>8</sup> Alternatively, the studies carried out on proline peptides<sup>9</sup> were only of limited success, because of their inherent ring-puckering. This makes it very difficult to interpret the stereoelectronic interactions arising from the chirality effect of residues neighboring the lactam moiety. Hence, a scaffold size reduction strategy intended to design “minimal” short peptide models becomes particularly attractive in terms of design simplicity and folding interpretation.

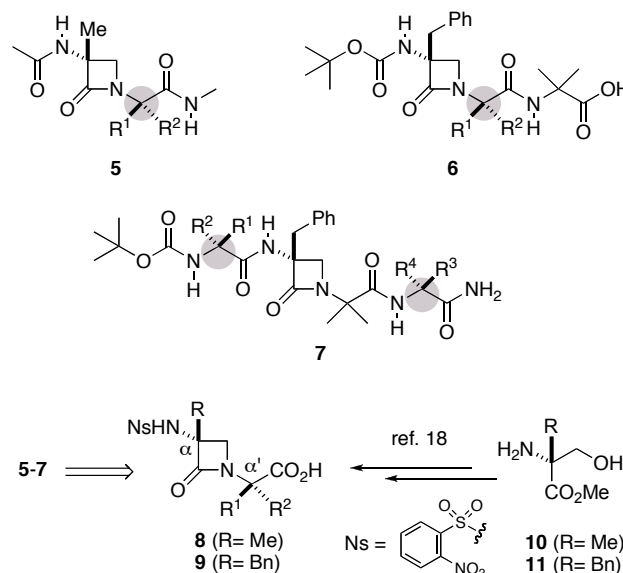
In our first accomplishment in this area<sup>10</sup> we introduced the  $\beta$ -lactam peptides **4** containing a central

1  $\alpha$ -alkyl- $\alpha$ -amino- $\beta$ -lactam ring placed as the ( $i+1$ ) residue.<sup>11</sup> This approach, termed “ $\beta$ -Lactam  
2 Scaffold-Assisted Design” ( $\beta$ -LSAD), involved the formal insertion of a single carbon atom ( $\text{C}\alpha\text{-}\underline{\text{H}} + \underline{\text{H}}\text{-}$   
3  $\text{N} \rightarrow \text{C}\alpha\text{-}\underline{\text{CH}_2}\text{-N}$ ) in the native peptide to form “minimal”  $\beta$ -lactam pseudopeptides **4**, which displayed a  
4 set of interesting structural features. Firstly, the linear disposition of the  $\text{C}\alpha$ , N and  $\text{C}\alpha'$  atoms within  
5 the  $\beta$ -lactam ring confers to peptidomimetics **4** a high potential for  $\beta$ -hairpin nucleation. Secondly, they  
6 possess a  $\psi_{(i+1)}$  dihedral angle fixed at  $120^\circ$  by the  $\beta$ -lactam ring, which prevents the formation of type-I  
7 and/or type-III  $\beta$ -turns (both with  $\psi_{(i+1)}$  near  $-30^\circ$ ) and favors only the  $\beta$ -II-turn/ $\gamma$ -turn conformational  
8 equilibrium.<sup>12</sup> Indeed, a preliminary conformational study of  $\beta$ -LSAD pseudopeptides containing a  
9 glycine ( $i+2$ ) residue (**4**;  $\text{R}^2 = \text{H}$ ) conducted in our laboratory confirmed their strong bias towards  
10 forming type-II  $\beta$ -turns which survive even in the presence of the highly coordinating solvent  
11 DMSO.<sup>10(a)</sup> Finally,  $\alpha$ -alkyl- $\alpha$ -amino- $\beta$ -lactam peptides are expected to be chemically much more stable  
12 than common  $\alpha$ -amino- $\beta$ -lactam antibiotics, which undergo easy ring-opening to  $\beta$ -amino acid  
13 derivatives. This transformation usually involves an  $\text{Ad}_\text{N}$  nucleophilic attack onto the  $\text{C}=\text{O}$   $\text{sp}^2$  carbon  
14 atom following the Bürgi-Dunitz trajectory.<sup>13</sup> In  $\beta$ -lactams **4** such a trajectory is hindered by the  $\alpha$ -  
15 substituents at both sides of the 2-azetidinone plane, thus diffculting the ring-opening reaction with  
16 respect to other  $\alpha$ -monosubstituted  $\beta$ -lactam analogues.<sup>14</sup> Recently, the surprisingly high “amidicity” of  
17 monocyclic  $\beta$ -lactams has also been invoked to account for their chemical stability.<sup>15</sup>

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41 As mentioned above, only the solution conformation of short  $\beta$ -lactam pseudopeptides containing a  
42 central  $-(\beta\text{-Lactam})\text{-(Gly)}-$  core has been studied so far and the possible  $\beta$ -turn stabilizing (or  
43 destabilizing) effect exerted by the chiral  $\alpha$ -amino acid residues neighboring the  $\beta$ -lactam nucleus  
44 remains to be explored. Herein we report the synthesis and detailed conformational analysis of three  
45 families of  $\beta$ -lactam pseudopeptide models designed to show the chiral effects of residues ( $i$ ) ( $i+2$ ) and  
46 ( $i+3$ ) on the folding of short  $\beta$ -lactam peptides **4** in solution and in the solid state. Finally, we further  
47 examine the ability of this motif to nucleate a  $\beta$ -hairpin, the shortest  $\beta$ -sheet structure.

## Results and Discussion

**Design and Synthesis of  $\beta$ -Lactam Pseudopeptide Models.** Short pseudopeptide models **5-7** (Figure 2) with homogeneous (*R*) configuration at the  $C_\alpha$  stereocenter of the  $\beta$ -lactam ring were selected for development because they are reminiscent of constrained natural L  $\alpha$ -amino acid residues.<sup>16</sup> Configurationally related  $\beta$ -lactam peptides **5** and **6** containing a single additional stereocenter at residue (*i*+2) were designed to carry terminal groups with different steric demands. Accordingly, mimetics **5** [Ac-( $\alpha$ -Me- $\beta$ -Lactam)-Aa-Me] represented the smallest capped  $\beta$ -lactam dipeptide structures containing three peripheral methyl groups, whereas mimetics **6** [Boc-( $\alpha$ -Bn- $\beta$ -Lactam)-Aa-Aib-OH] had a larger central benzyl group and bulkier achiral N- and C-terminal groups (Boc and Aib-OH). Finally, pseudopeptides **7** [Boc-Ala\*-( $\alpha$ -Bn- $\beta$ -Lactam)-Aib-Ala\*-NH<sub>2</sub>] were designed to ascertain the chiral effect of two additional stereocenters at the (*i*) and (*i*+3) alanine residues on the possible  $\beta$ -hairpin nucleation.

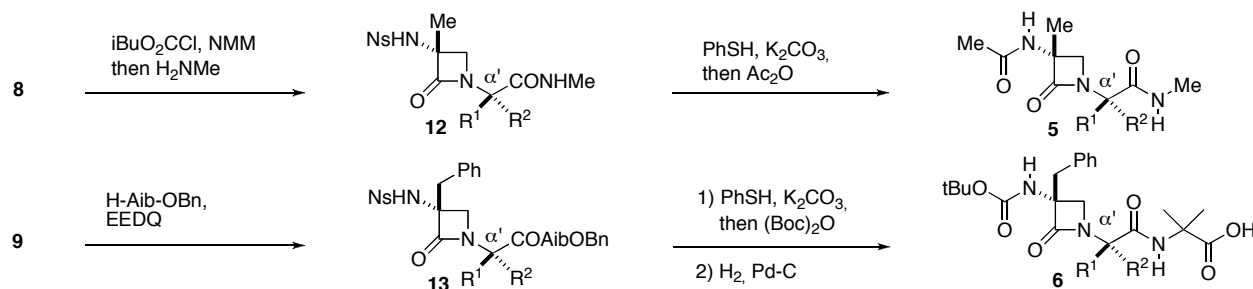


**Figure 2.** Top:  $\beta$ -Lactam pseudopeptide models **5-7** selected to study the chiral effect of residues (*i*), (*i*+2) and (*i*+3) on the peptide folding. Bottom: Synthesis of the  $\beta$ -lactam dipeptide scaffolds **8-9**.

1 The enantiopure scaffolds **8-9** required to form the central  $-(i+1)-(i+2)-$  pseudopeptide segments  
2 were easily obtainable from the corresponding  $\alpha$ -alkylserinates<sup>17</sup> **10-11** according to an operatively very  
3 simple method developed in our laboratory.<sup>18</sup> Tables 1 and 2 collect the details of the transformation of  
4 the dipeptide scaffolds **8, 9** and **14** into the target  $\beta$ -lactam pseudopeptide models.  
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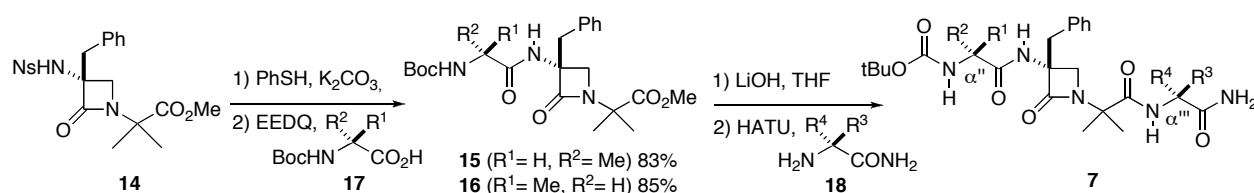
9  
10 As shown in Table 1, the synthesis of pseudopeptides **5** and **6** required the coupling of the C-terminal  
11 carboxylic acid group in **8** and **9** with methylamine and  $\alpha$ -aminoisobutyric benzyl ester, respectively.  
12 Formation of N-methylamides **12a-e** from non-hindered  $\beta$ -lactam carboxylic acid precursors was best  
13 performed using the mixed anhydride method assisted by isobutyl chloroformate and N-  
14 methylmorpholine, whereas couplings involving hindered Aib residues were most efficiently  
15 accomplished with EEDQ<sup>19</sup> reagent (products **13, 15, 16** and **7a-d**). Denosylation of compounds **12-13**  
16 proceeded smoothly with thiophenol<sup>20</sup> in the presence of potassium carbonate to afford the intermediate  
17  $\alpha$ -amino- $\beta$ -lactams, which were trapped in situ with acetic anhydride or di-*tert*-butyl dicarbonate to  
18 provide the desired mimetic models **5** or the analogs **6** after hydrogenolytic deprotection of the C-  
19 terminal benzyl ester. Stereochemical integrity was maintained in all transformations and no  
20 epimerization of the  $\alpha'$  stereocenter could be detected via NMR and HPLC analysis of the reaction  
21 crudes. Following a similar protocol (Table 2), mimetics **7** were obtained in good overall yields from the  
22  $\alpha',\alpha'$ -dimethyl- $\beta$ -lactam **14** after consecutive coupling with N-protected alanines **17** and alaninamides  
23 **18** using, respectively, the EEDQ and HATU/HOAT<sup>21</sup> reagents.  
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**Table 1.** Preparation of  $\beta$ -Lactam Peptidomimetics **5** and **6**.

$R^1$	$R^2$	Config. $C\alpha'$	Product	Yield (%)	Product	Yield (%)
H	H	----	<b>12a</b>	80	<b>5a</b>	84
Me	H	( <i>R</i> )	<b>12b</b>	58	<b>5b</b>	72
H	Me	( <i>S</i> )	<b>12c</b>	59	<b>5c</b>	81
Ph	H	( <i>R</i> )	<b>12d</b>	78	<b>5d</b>	89
H	Ph	( <i>S</i> )	<b>12e</b>	85	<b>5e</b>	94
Me	Me	----	<b>12f</b>	75 <sup>a)</sup>	<b>5f</b>	82
Me	H	( <i>R</i> )	<b>13b</b>	79	<b>6b</b>	79
H	Me	( <i>S</i> )	<b>13c</b>	70	<b>6c</b>	80
Ph	H	( <i>R</i> )	<b>13d</b>	55	<b>6d</b>	82
H	Ph	( <i>S</i> )	<b>13e</b>	66	<b>6e</b>	80
Me	Me	----	---	---	<b>6f</b> <sup>b)</sup>	92

<sup>a)</sup> EEDQ reagent was used as the amide formation reagent. <sup>b)</sup> The product was prepared from acid Boc-( $\beta$ -Lactam)-Aib-OH, which was coupled to H-Aib-OBn and submitted to hydrogenolysis.

**Table 2.** Preparation of  $\beta$ -Lactam Peptidomimetics **7**.

$R^1$	$R^2$	Config. $C\alpha''$	$R^3$	$R^4$	Config. $C\alpha'''$	Product	Yield <sup>a</sup> (%)
H	Me	( <i>R</i> )	H	Me	( <i>R</i> )	<b>7a</b>	80
H	Me	( <i>R</i> )	Me	H	( <i>S</i> )	<b>7b</b>	65
Me	H	( <i>S</i> )	Me	H	( <i>S</i> )	<b>7c</b>	73
Me	H	( <i>S</i> )	H	Me	( <i>R</i> )	<b>7d</b>	77

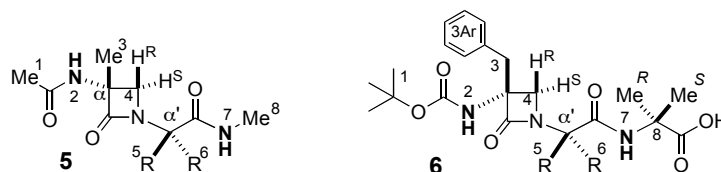
<sup>a)</sup> Overall yield from **15**, **16**.

**Solution Conformation of  $\beta$ -Lactam Peptides 5-6 with a Chiral ( $i+2$ ) Residue.** With the model  $\beta$ -lactam peptides in hand, we first addressed the NMR conformational analysis of the simplest families **5** [Ac-( $\alpha$ -Me- $\beta$ -Lactam)-Aa-Me] and **6** [Boc-( $\alpha$ -Bn- $\beta$ -Lactam)-Aa-Aib-OH] in order to characterize the presumably more populated  $\beta$ -II turn conformers in solution and to assess the possible stabilizing or destabilizing effects exerted by the chiral Aa residues on the conformer equilibrium. Although water was considered first as the most biologically meaningful medium for the study, finally DMSO<sub>d6</sub> solvent was chosen for practical reasons to ensure solubility. In the case of peptides **5a-f**, however, the recurrent overlapping of NH amide signals in dimethylsulfoxide precluded a straightforward interpretation of the results and CDCl<sub>3</sub> was used as solvent.

Solutions of each peptide **5a-f** and **6a-f** ( $5 \times 10^{-3}$  M) yielded sharp, well-resolved <sup>1</sup>H-NMR spectra consisting of single sets of signals in both solvents. All protons were unambiguously assigned from DQF-COSY and HMBC spectra (Supporting information Figures S1-S10). Chemical shift variations upon solvent change and <sup>1</sup>H-NMR thermal coefficients<sup>22</sup> for the exchangeable amide protons of the pseudopeptides in DMSO<sub>d6</sub> solutions were determined over a temperature range of 300-325K at 5K intervals (Table 3). An inspection of the data recorded immediately revealed a totally different deshielding for the  $\beta$ -lactam N<sub>2</sub>H amide protons and the N<sub>7</sub>H protons of the ( $i+3$ ) residue, suggesting the formation of a turned conformer in most of the cases with the N<sub>7</sub>H amide protons participating in the intramolecular hydrogen bond network of the  $\beta$ -lactam peptides and the N<sub>2</sub>H protons exposed to the solvent. This was evident from the analysis of thermal coefficients in DMSO<sub>d6</sub> (see Table 3, two right hand columns) showing absolute values in the range -2.3 to -4.9 ppb/K for N<sub>7</sub>H protons, whereas amides and carbamates N<sub>2</sub>H gave larger thermal coefficients (up to -8.9 ppb/K). Consistently, the solvent change from nonacceptor CDCl<sub>3</sub> to acceptor DMSO<sub>d6</sub> resulted in a very small chemical shift variation for N<sub>7</sub>H amides in all peptides (0.1 – 1.1 ppm), but in a significantly larger downfield shift (2.3 – 2.8 ppm) in N<sub>2</sub>H  $\beta$ -lactam amides. While seeking more insight into the conformational differences between the  $\beta$ -lactam peptide diastereomers with ( $i+2$ ) chiral  $\alpha$ -amino acid residues (Table 3, entries 2-

5 and 8-11), we noticed that the thermal coefficients of the  $N_7H$  protons were routinely larger for ( $i+2$ )-( $S$ ) diastereomers (**5c**, **5e** and **6c**, **6e**) than for ( $i+2$ )-( $R$ ) ones (**5b**, **5d** and **6b**, **6d**) and, furthermore, this effect was appreciably enhanced in pseudopeptides containing the bulkier phenylglycine residues (compare entries 4-5 and 10-11). This lower exposure of the ( $R$ ) diastereomers to the  $DMSO_{d6}$  coordinating solvent was consistent with the tight intramolecular  $N_7H...O=C$  hydrogen bonding of  $\beta$ -turned  $\beta$ -lactam peptides **4** (Figure 1), whereas the weaker hydrogen bonds of the ( $S$ ) diastereomers (entries 3, 5, 9 and 11) suggested a partial folding of the peptide backbone into  $\gamma$ -turned conformers or open conformers possessing more exposed amide protons. Finally, the  $\beta$ -lactam pseudopeptides with nonchiral Gly or Aib ( $i+2$ ) residues (entries 1, 6, 7 and 12) exhibited only very shielded  $N_7H$  protons in  $DMSO_{d6}$ , consistent with their likely participation in stable  $\beta$ -turns.

**Table 3.** Amide Proton  $^1H$ -NMR Chemical Shifts ( $\delta$ ) and Thermal Coefficients ( $\Delta\delta/\Delta T$ ) for  $\beta$ -Lactam Peptidomimetic Models **5a-f** and **6a-f**.



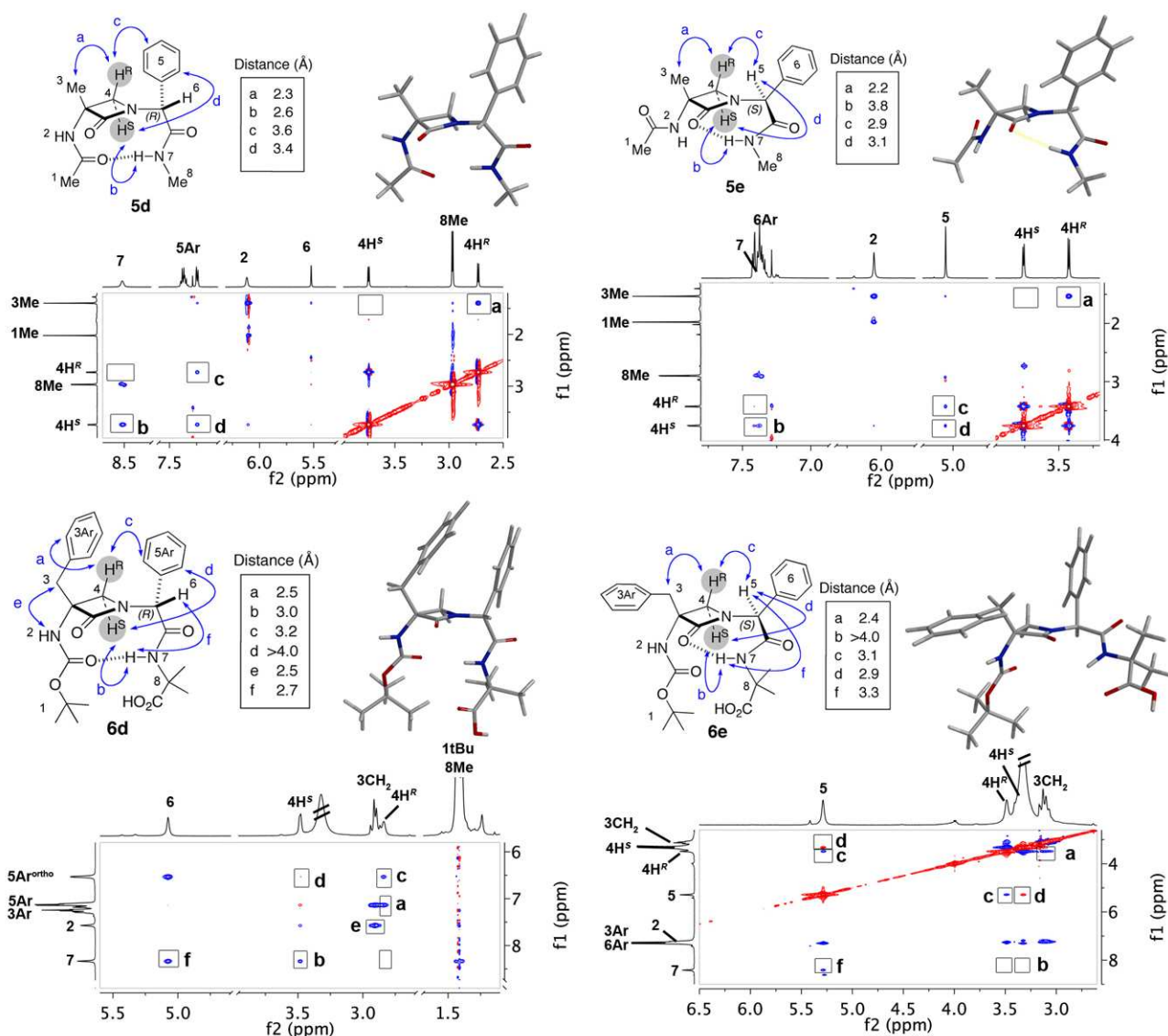
Entry	Comp	$R^5$	$R^6$	Config C $\alpha'$	$N_2H(\delta)^a$	$N_7H(\delta)^a$	$N_2H(\Delta\delta/\Delta T)^b$	$N_7H(\Delta\delta/\Delta T)^b$
1	<b>5a</b>	H	H	----	5.63 (+2.77)	8.09 (+0.08)	-5.3	-3.2
2	<b>5b</b>	Me	H	( $R$ )	5.60 (+2.72)	8.08 (+0.18)	-4.8	-3.4
3	<b>5c</b>	H	Me	( $S$ )	5.57 (+2.66)	7.94 (+0.29)	-4.5	-3.9
4	<b>5d</b>	Ph	H	( $R$ )	5.64 (+2.77)	8.41 (+0.16)	-5.0	-4.0
5	<b>5e</b>	H	Ph	( $S$ )	5.62 (+2.52)	7.12 (+1.15)	-4.5	-4.9
6	<b>5f</b>	Me	Me	----	5.55 (+2.81)	8.19 (+0.16)	-6.5	-3.9
7	<b>6a</b>	H	H	----	4.89 (+2.21)	7.82 (+0.01)	-8.3	-2.3
8	<b>6b</b>	Me	H	( $R$ )	4.76 (+2.84)	7.93 (+0.03)	-8.9	-2.8
9	<b>6c</b>	H	Me	( $S$ )	4.90 (+2.29)	7.83 (+0.10)	-9.0	-3.0
10	<b>6d</b>	Ph	H	( $R$ )	4.85 (+2.69)	8.18 (+0.14)	-8.3	-3.1
11	<b>6e</b>	H	Ph	( $S$ )	4.94 (+2.32) <sup>c</sup>	7.85 (+0.56)	-8.9	-4.2
12	<b>6f</b>	Me	Me	----	4.84 (+2.73)	7.76 (+0.10)	-8.6	-2.5

<sup>a</sup>Chemical shifts (ppm) measured in  $CDCl_3$ ; Values in parentheses represent variations of the chemical shift when the  $CDCl_3$  solvent was changed to  $DMSO_{d6}$ . <sup>b</sup>Thermal coefficients in ppb/K measured in  $DMSO_{d6}$ . <sup>c</sup>A mixture of conformers was observed at room temperature in  $CDCl_3$ , which coalesced in  $DMSO_{d6}$ .

Next, relevant noncontiguous inter-proton distances for peptides **5-6** were calculated following the ISPA (Isolated Spin-Pair Approximation) method<sup>23</sup> from the integration of key NOESY or ROESY cross-peak signals recorded at 300K (500MHz) at mixing times of 200-400 ms (Supporting information, Tables S1 and S2; Figures S15 and S16).

Some expansions of the NOESY spectra of **5d-e** (in CDCl<sub>3</sub>) and **6d-e** (in DMSO<sub>d6</sub>), incorporating a chiral phenylglycine (*i*+2) residue, are shown in Figure 3 as an example. The spectra gave rise to a number of NOE contacts, including the excellent diagnostic correlation peaks provided by the pro-*R* and pro-*S* diastereotopic C<sub>4</sub> protons placed, respectively, at the upper and lower faces of the β-lactam ring, as depicted in the figure. Only the 4H<sup>R</sup> proton gave a characteristic strong NOE contact (cross-peak **a**) with the 3Me singlet in peptides **5** and with the benzylic moiety 3/3Ar in peptides **6**. Conversely, the 4H<sup>S</sup> proton oriented to the lower face of the azetidin-2-one ring plane was used to monitor the 4H<sup>S</sup>–N<sub>7</sub>H distances (cross-peak **b**) and to estimate the preferred formation of canonical β-II turns (≈ 2.5–2.8 Å), distorted γ-turns (≈ 3.8–4.0 Å) or open conformers (> 4 Å). Additionally, the orientation of the R<sup>5</sup> and R<sup>6</sup> substituents of the chiral (*i*+2) residue relative to the β-lactam ring could be established on the basis of the distances of some of their key protons to 4H<sup>R</sup> and 4H<sup>S</sup> (cross-peaks **c** and **d**). In the case of **6d**, the abnormal shielding of 5Ar *ortho* protons (6.52 ppm) arising from the diamagnetic effect of the neighboring benzylic 3Ar ring provided a further indirect sign of β-turn conformation. Finally, as none of the peptides **5-6** gave measurable 4H<sup>R</sup>/N<sub>7</sub>H correlation cross-peaks, a significant participation in the conformational equilibrium of inverse γ<sup>i</sup>-turn β-lactam pseudopeptides with the (*i*+2)-(*i*+3) branch oriented toward the top side of the azetidinone ring was ruled out (see empty boxes in Figure 3).

From the inter-proton distances collected in Figure 3, a regular chiral effect trend could be observed for the β-turn/γ-turn equilibrium as the α'-(*R*) configuration of peptides **5d/6d** changed to α'-(*S*) in their diastereomers **5e/6e**. Indeed, the average 4H<sup>S</sup>–N<sub>7</sub>H distances (cross-peak **b**) were significantly shorter for **5d** and **6d** (2.6 and 3.0 Å) than for their respective isomers **5e** and **6e** (3.8 Å and >4 Å), suggesting

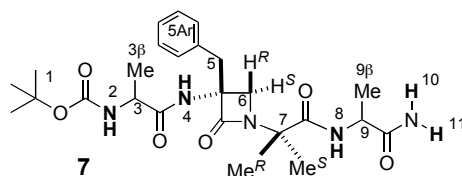


**Figure 3.** Expansion of the NOESY spectra of pseudopeptides **5d-e** in  $\text{CDCl}_3$  (top) and **6d-e** in  $\text{DMSO}_{d6}$  (bottom) showing a preferred  $\beta$ -II turn conformation (left hand) and a  $\gamma$ -turn conformation (right hand). Key NOE correlations with the upper ( $4\text{H}^R$ ) and lower ( $4\text{H}^S$ ) sides of the  $\beta$ -lactam ring plane are highlighted inside boxes. Empty boxes show the expected position of alternative **b** cross-peaks with  $4\text{H}^R$  protons. Model figures show energy-minimized SYBIL molecular mechanics structures restrained with NOESY inter-proton distances ( $\pm 0.3\text{\AA}$ ) (Supporting information Tables S1-S2).

that in the latter cases  $\beta$ -turns were weakened or broken in favor of  $\gamma$ -turns. This assumption was further supported by the close distances of the  $C\alpha H^5$  protons of the (*S*)-phenylglycine residue to  $4H^R$  and  $4H^S$  in **5e/6e** and also by the absence of a comparable interaction for  $C\alpha H^6$  in **5d/6d**. In line with these observations,  $\beta$ -lactam pseudopeptides **5b/6b** and **5c/6c** derived, respectively from (*R*)-alanine and (*S*)-alanine, showed similar  $\beta$ -turn destabilization trends upon (*i*+2)  $\alpha'$  stereocenter configuration inversion, albeit in these cases the chiral effect was less pronounced (Supporting information, Figures S15 and S16). Finally, all the  $\beta$ -lactam pseudopeptides with achiral (*i*+2) residues of glycine (**5a/6a**) or  $\alpha$ -aminoisobutyric acid Aib (**5f/6f**) populated exclusively very stable  $\beta$ -turn conformations and showed no tendency to form  $\gamma$ -turns.

**Solution Conformation of  $\beta$ -Lactam Peptides **7** with Chiral (*i*) and (*i*+3) Residues.** Following the same NMR methodology disclosed above, we next studied the conformational behavior of  $\beta$ -lactam peptidomimetics **7a-d** in  $DMSO_{d6}$  to check the chiral effect of (*i*) and (*i*+3) alanine residues on the nucleation of  $\beta$ -sheets. The thermal coefficient data collected in Table 4 show that all the (*i*+3)  $N_8H$  amide protons participate in strong intramolecular hydrogen bonds, likely forming stable  $\beta$ -II turns. Interestingly, unlike the “heterochiral” diastereomers **7b** and **7d**, each “homochiral” diastereomer

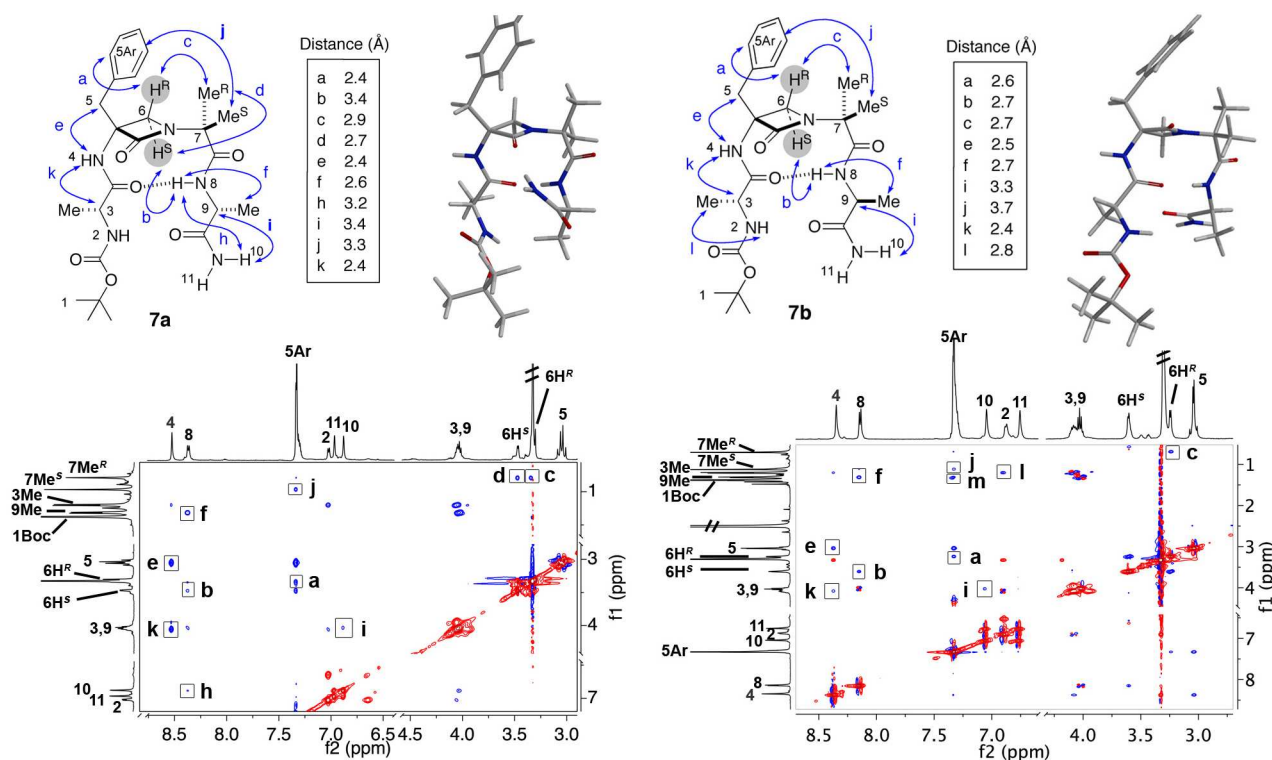
**Table 4.** Amide Proton  $^1H$ -NMR Thermal Coefficients ( $\Delta\delta/\Delta T$ )<sup>a</sup> for  $\beta$ -Lactam Peptidomimetic **7**.



Entry	Comp	Config (C <sub>3</sub> , C <sub>9</sub> )	N <sub>2</sub> H <sup>b</sup>	N <sub>4</sub> H	N <sub>8</sub> H	N <sub>10</sub> H <sup>b</sup>	N <sub>11</sub> H
1	<b>7a</b>	( <i>R</i> , <i>R</i> )	−7.4	−6.6	−2.3	−2.4	−5.2
2	<b>7b</b>	( <i>R</i> , <i>S</i> )	−7.3	−6.7	−2.8	−3.8	−4.1
3	<b>7c</b>	( <i>S</i> , <i>S</i> )	−6.3	−5.8	−3.5	−2.5	−5.2
4	<b>7d</b>	( <i>S</i> , <i>R</i> )	−7.0	−5.8	−2.5	−4.3	−4.5

<sup>a</sup> Thermal coefficients in ppb/K measured in  $DMSO_{d6}$ .

**7a** (*R,R*) and **7c** (*S,S*) showed an additional solvent-shielded N<sub>10</sub>H terminal *trans*-amide proton, which we hoped would participate as donors of the Boc group in  $\beta$ -hairpin type secondary structures. A more detailed examination of the inter-proton distances from ROESY spectra of **7a**, however, revealed that the actual acceptor of the N<sub>10</sub>H amide proton was the  $\beta$ -lactam carbonyl oxygen, thus forming two overlapped  $\beta$ -II/ $\beta$ -I turns (Figure 4). In particular, this assumption was further supported by the long range N<sub>8</sub>H–N<sub>10</sub>H NOE diagnostic cross-peak **h** observed for the homochiral peptide **7a**, but not for the heterochiral peptide **7b** (for an analogous behavior for diastereomers **7c**/**7d** see Supporting information Figure S17).

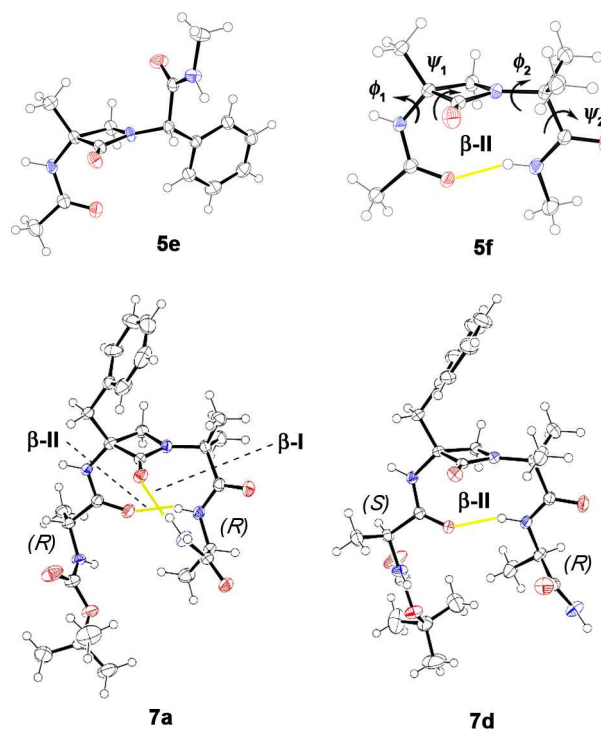


**Figure 4.** Expansion of the ROESY spectra of pseudopeptides **7a** and **7b** in DMSO<sub>d6</sub> showing two overlapped  $\beta$ -turns sharing the  $\beta$ -lactam ring and the N<sub>8</sub>H–N<sub>10</sub>H close distance NOE correlation (**h**). The model figure shows the energy-minimized structure restrained with ROESY inter-proton distances ( $\pm 0.3\text{\AA}$ ) (Supporting information Table S3)

**Solid State Conformation of  $\beta$ -Lactam Peptidomimetics 5-7.** Single crystals of the  $\beta$ -lactam pseudopeptides **5e**, **5f**, **7a** and **7d** were grown from their solutions in MeOH and were used for low-temperature X-ray structure determination (Figure 5). A detailed analysis of some key structural parameters, Table 5, revealed the formation of well defined  $\beta$ -II turn conformers in all instances, with the exception of the (*S*)-phenylglycine-derived mimetic **5e**, which showed an extended backbone structure with the (*i*) acetamido group and the (*i*+3) phenylglycine N-methylcarbamoyl group lying on opposite sides of the plane of the  $\beta$ -lactam ring. Consistent with the extremely destabilized  $\gamma$ -turn conformation observed for this pseudopeptide in solution by NMR, no intramolecular hydrogen bond was detected in the solid state. Instead, each NH amide group acted as a donor to form intermolecular bifurcated hydrogen bonds with the C=O oxygen atom of the  $\beta$ -lactam ring.

Mimetic models **5f**, **7a** and **7d** containing the (*i*+2) Aib residue fulfilled all the structural criteria of canonical type-II  $\beta$ -turns in terms of  $_{(i)}\text{C}=\text{O}\cdots\text{HN}_{(i+3)}$  hydrogen bond distances (1.9–2.1 Å),  $_{(i)}\text{O}\cdots\text{H}-\text{N}_{(i+3)}$  angles (159°–167°) and the  $\delta$  pseudodihedral angle formed by the four  $\text{C}\alpha$  atoms (5°–14°), which was very close to the ideal null value. Only a slight distortion (15–20°) caused by the coplanar arrangement of  $\text{N}_{(i+1)}$ ,  $\text{C}\alpha_{(i+1)}$ ,  $\text{N}_{(i+2)}$  and  $\text{C}\alpha_{(i+2)}$  atoms around the central  $\beta$ -lactam ring, was observed for the torsion angles  $\phi$  -  $\psi$  in residues (*i*+1)-(*i*+2) with respect to the ideal values for a type-II  $\beta$ -turn. On the other hand, none of the peptides **7a** and **7d** showed a second hydrogen bond between the terminal Boc and  $\text{NH}_2$  groups denoting the formation of  $\beta$ -sheet structures. In spite of that, the  $\text{NH}_{\text{trans}}$  terminal amide proton of peptide **7a** acted as a donor to the  $\beta$ -lactam carbonyl oxygen atom to form a quasycanonical  $\beta$ -I turn partially overlapping the  $\beta$ -II turn. To the best of our knowledge, this is the first example of such a type of double motif in solid pseudopeptide structures.<sup>24</sup>





**Figure 5.** ORTEP plots (50% probability ellipsoids) of the molecular structures of  $\beta$ -lactam peptidomimetics **5e**, **5f**, **7a** and **7d** in the solid state. Compound **7a** shows overlapping  $\beta$ -II/ $\beta$ -I turns.

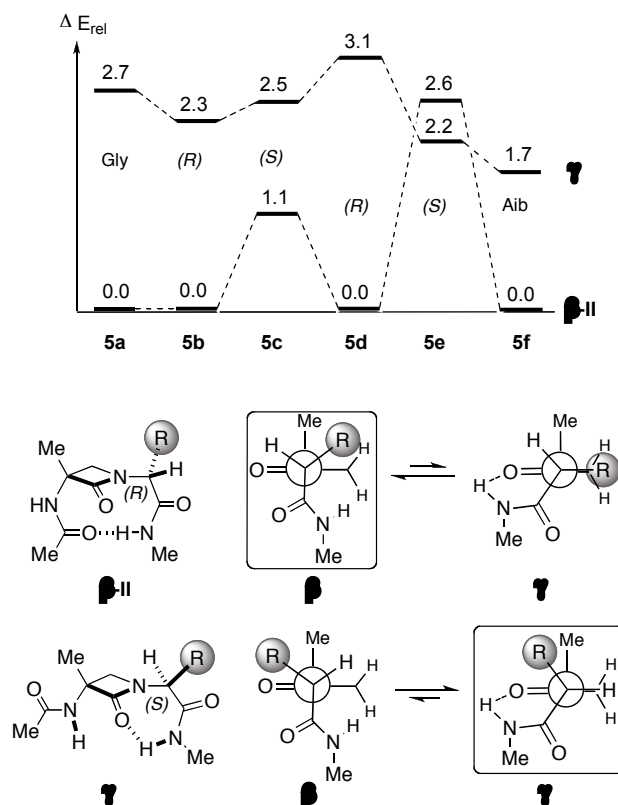
**Table 5.** Selected Geometric Parameters for  $\beta$ -Lactam Peptidomimetic Models **5e**, **5f**, **7a** and **7d**.

Comp	${}_{(i)}\text{C}=\text{O}\cdots\text{HN}_{(i+3)}$ ( $\text{\AA}$ )	$\phi_{(i+1)}; \psi_{(i+1)}$ ( $^\circ$ )	$\phi_{(i+2)}; \psi_{(i+2)}$ ( $^\circ$ )	$\delta$ ( $^\circ$ ) <sup>a</sup>	${}_{(i)}\text{C}=\text{O}\cdots\text{H}-\text{N}_{(i+3)}$ ( $^\circ$ ) <sup>b</sup>
<b>5e</b>	> 4.0	-39.0(4); 126.6(3)	-95.2(4); 134.9(3)	-157.0	---
<b>5f</b>	1.92(3)	-38.3(2); 125.4(2)	90.8(2); -2.8(2)	14.4	160(3)
<b>7a</b> ( $\beta$ -II)	2.07(3)	-39.7(3); 118.9(2)	47.0(4); 30.4(3)	-31.3	167(2)
<b>7a</b> ( $\beta$ -I)	1.87(4)	47.0(4); 30.4(3)	67.2(3); 24.3(3)	-77.3	168(5)
<b>7d</b>	1.97(3)	-40.6(3); 123.9(2)	95.4(3); -2.5(3)	15.9	159(3)
$\beta$ -II (ideal)	1.8– 2.5	-60; 120	80; 0	0	160
$\beta$ -I (ideal)	1.8– 2.5	60; 30	90; 0	0	160
$\gamma$ (ideal)	1.8– 2.5	---; ---	50; -60	0	160

<sup>a</sup>Pseudodihedral angle formed by the four  $\text{C}\alpha$  atoms of the peptide models. <sup>b</sup> $\text{O}\cdots\text{H}-\text{N}$  angle.

Finally, a rationale for the chiral effect caused by the  $(i+2)$  residue on the relative stability of  $\beta$ -II/ $\gamma$  conformers in pseudopeptides **5** was established on the basis of the steric interactions of  $\text{R}^1$  and  $\text{R}^2$

groups with the carbonyl and methylene groups of the  $\beta$ -lactam ring ( $i+1$ ). Accordingly, we submitted structures **5a-f** to a DFT computational optimization<sup>25</sup> consisting of a Monte Carlo search (6-31G\*), followed by structure minimization at the B3LYP/6-31++G\*\* level.<sup>26</sup> Energy values collected in Figure 6 show a general trend for the preferred stabilization (2-3 Kcal/mol) of type II  $\beta$ -turns, over the normal  $\gamma$ -turns. As expected, no type-I  $\beta$ -turn could be characterized as a stable energy minimum. In addition, a clear chiral-effect destabilizing the  $\beta$ -II turn was apparent in the case of heterochiral ( $R,S$ ) mimetics **5c** and **5e** with respect to the homochiral ( $R,R$ ) mimetics **5b** and **5d** and this energy-difference increased when the size of R group was increased from methyl to phenyl. In the latter case, a dramatic inversion in the stability of the  $\gamma$ -turn is also observed. Finally, models **5a** and **5f** bearing respectively achiral ( $i+2$ ) residues of Gly and Aib, displayed very similar relative stability patterns favoring the type  $\beta$ -II conformer over the  $\gamma$ -turns, albeit in the later case the energy-difference was lower in the latter case.



**Figure 6.** Top: relative zero-energies (Kcal·mol<sup>-1</sup>) of  $\beta$ -II turn and  $\gamma$ -turn conformers for pseudopeptides **5a-5f**. Bottom: steric interactions accounting for the chiral-effect and relative conformer stabilities. The

energy values for the isomer pairs **5b-c** and **5d-e** are referred to the lower energy conformer in each case.

Figure 6 also depicts the Newman projections of models **5** with the  $\beta$ -lactam nitrogen atom and  $C\alpha'$  eclipsed. In homochiral (*R,R*) isomers the group R can adopt an alternative disposition only when the  $\beta$ -turn-II conformation is reached, leading to the more stable conformations. The exception to this general bias only occurs when the (*i*+2) residue is heterochiral with respect to the  $\beta$ -lactam (*i*+1) stereocenter and the size-difference between R and the  $\beta$ -lactam  $\alpha$ - group is large. For instance, in model **5e** with a (*S*)-phenylglycine (*i*+2) residue, conformer  $\beta$ -II is destabilized by the steric repulsion of the phenyl group and the  $\beta$ -lactam carbonyl oxygen atom, resulting in the stabilization of the  $\gamma$ -turn conformer.

## Conclusions

The present work illustrates the intrinsic ability of short  $\beta$ -lactam peptide chains to form  $\beta$ -II and  $\gamma$ -turn structures in solution. It also emphasizes that “one carbon-scaffolded”  $\beta$ -lactam pseudopeptides provide very precise information on the H-bonding pattern in short peptide models, which complements prior quantum chemistry and gas-phase laser-desorbed experiments.<sup>25(c)</sup> 2D NMR experiments conducted in solution have provided compelling evidence that  $\beta$ -lactam pseudopeptides **5-6** containing a central homochiral  $-(R)\text{-}\beta\text{-Lactam-}(R)\text{-Aa}-$  segment behave as betagenic, whereas the heterochiral analogues  $-(R)\text{-}\beta\text{-Lactam-}(S)\text{-Aa}-$  destabilize  $\beta$ -turn conformations and favor  $\gamma$ -turned peptides, especially when the (*i*+2) Aa residue carries sterically demanding  $C\alpha$  backbone substituent groups. In addition, pseudopeptides **7** containing a central betagenic  $-(R)\text{-}\beta\text{-Lactam-Aib}-$  nucleus flanked by heterochiral (*i*) and (*i*+3)  $\alpha$ -amino acid residues do not show a straightforward ability to stabilize  $\beta$ -hairpins. Instead, the homochiral counterparts show a strong tendency to form unprecedented  $\beta$ -II/ $\beta$ -I overlapped double turns around the central  $\beta$ -lactam core.

In the light of the current results, it can be stated that chirality-driven conformational effect may

1 ultimately determine the preferred type of turn stabilized by  $\beta$ -lactam pseudopeptides. Therefore, the  $\beta$ -  
2  
3 LASD design model, consisting in the incorporation of an intra-residual methylene bridge to frozen  
4  
5 type-II  $\beta$ -turns, must be applied cautiously to “all-L” peptide natural segments when chiral residues  
6  
7 neighboring the central  $\beta$ -lactam core are involved. Despite this limitation, the  $\beta$ -LASD design method  
8  
9 provides a simple steric model that opens up the route for a better understanding of the steric  
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11 interactions driving the shallow  $\beta$ -/ $\gamma$ - transition in larger peptides.  
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## Experimental Section

**General Experimental Section.** All reactions were carried out under an atmosphere of nitrogen in oven or flame-dried glassware with magnetic stirring. Solvents were distilled prior to use. Anhydrous tetrahydrofuran (THF) and dichloromethane were distilled, respectively, from sodium metal/benzophenone ketyl and from calcium hydride. Reaction products were purified by flash chromatography using silica gel 60 (230-400 mesh). Analytical TLC was performed on 0.25 mm silica gel 60-F plates and spots were visualized, either with UV light or with the spray reagent phosphomolybdic acid-ammonium cerium (IV) nitric-sulfuric acid-water, followed by heating. Melting points are uncorrected.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded at 500 MHz and 125.7 MHz respectively and are reported as  $\delta$  values (ppm) relative to residual  $\text{CDCl}_3$  ( $\delta$  7.26), DMSO ( $\delta$  2.49) and  $\text{CDCl}_3$  ( $\delta$  77.0), DMSO ( $\delta$  39.5) as internal standards. Mass spectra were obtained from samples ionized under EI (70 eV) or CI conditions and detected using a quadrupole or a TOC mass analyzer, after direct injection (HRMS) or GC-MS coupling (column: fused silica gel, 15 m, 0.25 mm, 0.25 nm phase SPB-5). The preparations and spectroscopic data of compounds **6a**, **8a**, **9a-f** and **14** were reported previously.<sup>10(a), 18</sup>

**General Procedure for the Preparation of 1-Carboxyalkyl-3-methyl-3-(2-nitrobenzenesulfonylamino)azetidin-2-ones 8b-f:**<sup>18</sup> To a solution of (*R*) methyl 2-methyl-serinate **10** (1.90 mmol, 0.32 g) and 2-nitrobenzenesulfonyl chloride ( $\text{NsCl}$ , 4.18 mmol, 0.935 g) in acetonitrile (30 mL) was added  $\text{KHCO}_3$  (9.50 mmol, 0.951 g) and the suspension was stirred at reflux for 16 hours. Then, saturated aqueous  $\text{NaHCO}_3$  (15 mL) was added and the mixture was extracted with EtOAc (3 x 15 mL). The organic layer was dried ( $\text{MgSO}_4$ ) and the solvents were evaporated at reduced pressure to give pure (*2R*)-2-methyl-2-methoxycarbonyl-1-(2-nitrobenzenesulfonyl)-aziridine. A solution of the aziridine (1.51 mmol) and the corresponding  $\beta$ -(*tert*-butyldimethylsilyloxy)-ethylamine (1.51 mmol) in dry

acetonitrile (30 mL) was stirred at room temperature for 20 hours. Then saturated aqueous NaHCO<sub>3</sub> (15 mL) was added and mixture was extracted with EtOAc (3 x 15 mL), the organic fractions were dried (MgSO<sub>4</sub>) and filtered, and solvents were evaporated in vacuo to give the corresponding β-azaalanine, which was purified by column chromatography (hexanes/EtOAc 4:1). To a stirred solution of the β-azaalanine (1 mmol) in dry THF (15 mL), 2.5 mmol of LiHMDS in THF (1M) were added. After stirring for 12 hours, the mixture was washed with a saturated aqueous solution of NaHCO<sub>3</sub> (3x10 mL) and the aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The solvent was evaporated under reduced pressure and the crude product was purified by column chromatography (hexanes/EtOAc, 1:7). The intermediate obtained was oxidated following two different procedures:

**Oxidation Method A:**<sup>27</sup> BAIB (1.069 mmol, 0.344 g), TEMPO (0.243 mmol, 0.038 g), and the corresponding *N*-hydroxyethyl-β-lactam (0.486 mmol) were combined in the reaction vessel, and to this mixture was added 1:1 acetone-water solution (2 mL). The reaction mixture was stirred for 3h before evaporation of the solvents under reduce pressure. The resulting product was purified by column chromatography (hexanes/EtOAc, 1:10 or CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 10:1).

**Oxidation Method B:** The Jones reagent<sup>28</sup> (1.48 mmol) was added drop-wise to the solution of the corresponding *N*-hydroxyphenylethyl-β-lactam (0.74 mmol) in acetone (10 mL) at 0°C and the mixture was stirred at the same temperature for 2h. The oxidant excess was quenched with <sup>i</sup>PrOH (10 mL) and the dark solid formed was dissolved with H<sub>2</sub>O (4 mL). The mixture was extracted with EtOAc (3 x 10 mL) and the combined organic fractions were washed with brine (5 mL). The organic solution was dried (MgSO<sub>4</sub>), evaporated under reduced pressure, and the resulting product was purified by column chromatography (MeOH/CH<sub>2</sub>Cl<sub>2</sub>, 1:10).

**(3*R*)-1-[(*R*)-1-Carboxyethyl]-3-methyl-3-(2-nitrobenzenesulfonylamino)azetidin-2-one (8b):** The general procedure was followed (Oxidation method A) from *N*-[(2*R*)-2-hydroxy-1-methylethyl]-β-lactam (0.524 mmol, 0.180 g). Yield: 0.160 g (87%); yellowish solid; m.p. 55°C; [α]<sub>D</sub><sup>25</sup> +7.7 (0.45, MeOH); IR (KBr) ν<sub>max</sub> 3320, 2944, 1747, 1718, 1545, 1169, 832 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) δ<sub>H</sub>

8.22–7.78 (m, 4H), 4.09 (q, 1H,  $J = 7.2$  Hz), 3.72 (d, 1H,  $J = 5.8$  Hz), 3.35 (d, 1H,  $J = 5.8$  Hz), 1.52 (s, 3H), 1.38 (d, 3H,  $J = 7.3$  Hz).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  177.7, 169.4, 149.4, 136.4, 135.0, 133.7, 131.9, 125.9, 66.5, 54.8, 54.3, 20.5, 16.5. MS  $m/z$  (ESI, negative polarity): MS-1: 355.9; MS2(355.9): 308.8, 200.7, 185.7, 137.8. Anal. calcd. for  $\text{C}_{13}\text{H}_{15}\text{N}_3\text{O}_7\text{S}$ : C, 43.69; H, 4.23; N, 11.76. Found: C, 43.50; H, 4.17; N, 11.49.

**(3R)-1-[(S)-1-Carboxyethyl]-3-methyl-(2-nitrobenzenesulfonylamino)azetidin-2-one (8c):** The general procedure was followed (Oxidation method A) from *N*-hydroxy-(2*S*)-methylethyl- $\beta$ -lactam (0.350 mmol, 0.120 g). Yield: 0.110 g (88%); white solid; m.p. 58–60°C;  $[\alpha]_{\text{D}}^{25} -21.3$  (0.5, MeOH). IR (KBr)  $\nu_{\text{max}}$  3260, 2901, 1742, 1728, 1543, 1170, 820  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta_{\text{H}}$  8.17–7.83 (m, 4H), 4.23 (q, 1H,  $J = 7.5$  Hz), 3.63 (d, 1H,  $J = 5.5$  Hz), 3.50 (d, 1H,  $J = 5.5$  Hz), 1.58 (s, 3H), 1.38 (d, 3H,  $J = 7.5$  Hz).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  176.1, 168.1, 147.9, 135.1, 133.6, 132.2, 130.1, 124.4, 65.0, 52.5, 51.5, 19.3, 14.9. MS  $m/z$  (ESI, negative polarity): MS-1: 356.0; MS2(356.0): 200.7, 137.8; MS3(200.7): 136.8. Anal. calcd. for  $\text{C}_{13}\text{H}_{15}\text{N}_3\text{O}_7\text{S}$ : C, 43.69; H, 4.23; N, 11.76. Found: C, 43.54; H, 4.46; N, 11.47.

**(3R)-3-Methyl-3-(2-nitrobenzenesulfonylamino)-1-[(R)-1-phenyl-carboxymethyl]azetidin-2-one (8d):** The general procedure was followed (Oxidation method B) from *N*-hydroxy-(2*R*)-phenylethyl- $\beta$ -lactam (1.43 mmol, 0.58 g). Yield: 0.330 g (55%); yellowish powder; m.p. 115°C;  $[\alpha]_{\text{D}}^{25} 56.1$  (0.7, MeOH); IR (KBr)  $\nu_{\text{max}}$  3050, 2934, 1749, 1725, 1530, 1156, 802  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta_{\text{H}}$  8.24–7.32 (m, 9H), 5.37 (s, 1H), 3.97 (d, 1H,  $J = 6.0$  Hz), 3.08 (d, 1H,  $J = 6.0$  Hz), 1.41 (s, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  170.9, 166.4, 146.4, 134.2, 132.2, 130.9, 129.1, 126.9, 126.5, 126.4, 123.1, 63.8, 58.4, 52.5, 17.8. MS  $m/z$  (ESI, positive polarity): MS+23: 442.0, 420.0; MS2(420.1): 392.0; 204.9; MS3(392.0): 346.0, 205.0, 158.9. Anal. calcd. for  $\text{C}_{18}\text{H}_{17}\text{N}_3\text{O}_7\text{S}$ : C, 51.55; H, 4.09; N, 10.02. Found: C, 51.78; H, 4.28; N, 9.76.

**(3R)-3-Methyl-3-(2-nitrobenzenesulfonylamino)-1-[(S)-1-phenyl-carboxymethyl]azetidin-2-one (8e):** The general procedure was followed (Oxidation method B) from *N*-hydroxy-(2*S*)-phenylethyl- $\beta$ -

lactam (0.740 mmol, 0.300 g). Yield: 0.180 g (58%); yellowish powder; m.p. 188-190°C;  $[\alpha]_D^{25} +66.3$  (1.25, MeOH); IR (KBr)  $\nu_{\max}$  3360, 2944, 1741, 1736, 1536, 1167, 826  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta_{\text{H}}$  7.83–7.33 (m, 9H), 5.38 (s, 1H), 3.56 (d, 1H,  $J = 5.6$  Hz), 3.23 (d, 1H,  $J = 5.6$  Hz), 1.60 (s, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  173.4, 168.1, 147.8, 135.6, 134.9, 133.6, 132.2, 130.0, 128.4, 128.3, 127.7, 124.5, 65.0, 59.8, 52.8, 19.7. MS  $m/z$  (ESI, positive polarity): MS+23: 442.0, 420.1; MS2(420.1): 392.0; MS3(392.0): 346.0, 205.0, 159.0. Anal. calcd. for  $\text{C}_{18}\text{H}_{17}\text{N}_3\text{O}_7\text{S}$ : C, 51.55; H, 4.09; N, 10.02. Found: C, 51.32; H, 3.82; N, 9.82.

**(3R) 1-(1-Carboxy-isopropyl)-3-methyl-3-(2-nitrobenzenesulfonylamino)azetidin-2-one (8f):** To the solution of *N*-(methoxycarbonyl-isopropyl)- $\beta$ -lactam (0.259 mmol, 0.110 g) in THF (1 mL) was added a solution of  $\text{LiOH}\cdot\text{H}_2\text{O}$  (1.299 mmol, 0.055 g) in  $\text{H}_2\text{O}$  (1 mL). The reaction mixture was stirred at room temperature for 2h and then was acidified with 2M HCl and extracted with EtOAc (3 x 5 mL). The organic layer was dried over  $\text{MgSO}_4$  and evaporated to give a product which was purified by column chromatography (hexanes/EtOAc, 2:1). Yield: 0.090 g (93%); yellow oil;  $[\alpha]_D^{25} -33.0$  (1.2, MeOH); IR (KBr)  $\nu_{\max}$  3260, 2989, 1785, 1764, 1532, 1179, 816  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta_{\text{H}}$  8.16–7.78 (m, 4H), 3.68 (d, 1H,  $J = 5.0$  Hz), 3.34 (d, 1H,  $J = 5.0$  Hz), 1.61 (s, 3H), 1.52 (s, 3H), 1.44 (s, 3H).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  175.9, 169.1, 149.3, 136.4, 135.0, 133.6, 131.7, 125.9, 65.6, 60.2, 54.5, 24.3, 24.2, 20.9. MS  $m/z$  (ESI, negative polarity): MS-1: 383.0; MS2(383.0): 200.7, 185.7, 137.8; MS3(137.8): 117.9. Anal. calcd. for  $\text{C}_{14}\text{H}_{17}\text{N}_3\text{O}_7\text{S}$ : C, 45.28; H, 4.61; N, 11.32. Found: C, 44.97; H, 4.77; N, 11.53.

**General Procedure for the Preparation of 3-Methyl-1-[(*N*-methylcarbamoyl)alkyl]-3-(2-nitrobenzenesulfonylamino)azetidin-2-ones (12a-f):** To the solution of the corresponding  $\beta$ -lactam **8** (0.175 mmol, 0.060 g) in THF (1.5 mL) NMM (0.175 mmol, 0.020 mL) and IBCF<sup>29</sup> (0.175 mmol, 0.022 mL) were successively added at  $-20^\circ\text{C}$ . After an activation period of 3 min, 40% aqueous methylamine (0.350 mmol, 0.012 mL) was added and the resulting solution was stirred for 45 min at  $-20^\circ\text{C}$ . After the



solvent was evaporated under reduced pressure, the residue was directly loaded on silica gel (hexanes/EtOAc, 1:7) to give the corresponding *N*-methylcarbamoyl- $\beta$ -lactam.

**(3*R*)-3-Methyl-1-(*N*-methylcarbamoyl-methyl)-3-(2-nitrobenzenesulfonylamino)azetidin-2-one**

**(12a):** The general procedure was followed from  $\beta$ -lactam (**8a**) (0.175 mmol, 0.060 g). The obtained crude product was purified by preparative TLC (hexanes/EtOAc, 1:7). Yield: 0.050 g (80%); white powder; m.p. 145-147°C;  $[\alpha]_D^{25}$  -87.3 (1.5, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr)  $\nu_{\max}$  3108, 2990, 1760, 1732, 1526, 1140, 825 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta_H$  8.10–7.26 (m, 4H), 6.98 (br s, 1H), 5.95 (s, 1H), 4.35 (d, 1H, *J* = 17.0 Hz), 3.89 (d, 1H, *J* = 5.5 Hz), 3.57 (d, 1H, *J* = 17.0 Hz), 3.37 (d, 1H, *J* = 5.5 Hz), 2.90 (d, 3H, *J* = 5.5 Hz), 1.62 (s, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta_C$  167.4, 167.1, 147.6, 134.9, 133.9, 133.2, 130.8, 125.5, 67.9, 57.4, 45.6, 26.3, 21.8. MS *m/z* (ESI, negative polarity): MS-1: 354.9; MS2(354.9): 135.8 137.7 185.7, 193.7. Anal. calcd. for C<sub>13</sub>H<sub>16</sub>N<sub>4</sub>O<sub>6</sub>S: C, 43.82; H, 4.53; N, 15.72. Found: C, 43.80; H, 4.69; N, 15.47.

**(3*R*)-3-Methyl-1-[(1*R*)-1-(*N*-methylcarbamoyl)-ethyl]-3-(2-nitrobenzenesulfonylamino)-azetidin-**

**2-one (12b):** The general procedure was followed from  $\beta$ -lactam (**8b**) (0.08 mmol, 0.030 g). The crude product was purified by preparative TLC (hexanes/EtOAc, 1:7). Yield: 0.018 g (58%); white solid; m.p. 152°C;  $[\alpha]_D^{25}$  -67.3 (0.75, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr)  $\nu_{\max}$  3150, 2963, 1750, 1735, 1520, 1160, 825 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta_H$  8.09–7.74 (m, 4H), 7.08 (br s, 1H), 5.92 (s, 1H), 4.47 (q, 1H, *J* = 7.3 Hz), 3.74 (d, 1H, *J* = 5.5 Hz), 3.40 (d, 1H, *J* = 5.5 Hz), 2.88 (d, 3H, *J* = 4.7 Hz), 1.60 (s, 3H), 1.42 (d, 3H, *J* = 7.3 Hz); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta_C$  170.1, 166.9, 147.6, 135.0, 133.9, 133.2, 130.8, 125.4, 66.5, 53.9, 50.3, 26.4, 21.9, 14.5. MS *m/z* (ESI, negative polarity): MS-1: 369.0; MS2(369.0): 185.7, 137.8, 126.8. Anal. calcd. for C<sub>14</sub>H<sub>18</sub>N<sub>4</sub>O<sub>6</sub>S: C, 45.40; H, 4.90; N, 15.11. Found: C, 45.41; H, 4.78; N, 15.20.

**(3*R*)-3-Methyl-1-[(1*S*)-1-(*N*-methylcarbamoyl)-ethyl]-3-(2-nitrobenzenesulfonylamino)-azetidin-**

**2-one (12c):** The general procedure was followed from  $\beta$ -lactam (**8c**) (0.078 mmol, 0.028 g). The crude product was purified by preparative TLC (hexanes/EtOAc, 1:7). Yield: 0.017 g (59%); white solid; m.p. 163–164°C;  $[\alpha]_D^{25}$  -69.3 (0.67, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr)  $\nu_{\max}$  3120, 2959, 1765, 1745, 1540, 1100, 808 cm<sup>-1</sup>;

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> 8.12–7.72 (m, 4H), 6.56 (br s, 1H), 5.96 (s, 1H), 4.06 (q, 1H, *J* = 7.3 Hz), 3.84 (d, 1H, *J* = 5.5 Hz), 3.39 (d, 1H, *J* = 5.5 Hz), 2.84 (d, 3H, *J* = 4.8 Hz), 1.58 (s, 3H), 1.57 (d, 3H, *J* = 7.3 Hz); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ<sub>C</sub> 171.1, 167.4, 148.0, 135.8, 134.2, 133.5, 131.2, 125.8, 66.5, 55.9, 53.9, 26.8, 21.9, 15.9. MS *m/z* (ESI, negative polarity): MS-1: 369.0; MS2(369.0): 185.7, 137.8, 126.9; MS3(137.0): 121.8, 90.0. Anal. calcd. for C<sub>14</sub>H<sub>18</sub>N<sub>4</sub>O<sub>6</sub>S: C, 45.40; H, 4.90; N, 15.11. Found: C, 45.04; H, 4.88; N, 15.23.

**(3R)-3-Methyl-1-[(1R)-1-(N-methylcarbamoyl)-1-phenyl-methyl]-3-(2-**

**nitrobenzenesulfonylamino)-azetidin-2-one (12d):** To a solution of β-lactam (**8d**) (0.24 mmol, 0.100 g) and methylamine (2M in THF; 0.357 mmol, 0.179 mL) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) were added sequentially DCC (0.334 mmol, 0.069 g) and HOBT (0.286 mmol, 0.039 g) at 0°C. The reaction mixture was left to warm up and stirred at room temperature for 12h. Then solvent was removed under reduced pressure and the resulting crude product was purified by preparative TLC (hexanes/EtOAc, 1:5). Yield: 0.080 g (78%); yellowish powder; m.p. 145°C; [α]<sub>D</sub><sup>25</sup> -144.4 (0.75, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr) ν<sub>max</sub> 3320, 2984, 1768, 1732, 1540, 1158, 828 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> 8.14–7.25 (m, 10H), 5.52 (s, 1H), 3.80 (d, 1H, *J* = 5.8 Hz), 2.99 (d, 3H, *J* = 4.7 Hz), 2.94 (d, 1H, *J* = 5.8 Hz), 1.46 (s, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ<sub>C</sub> 169.1, 167.2, 147.9, 135.4, 134.3, 133.7, 131.3, 129.6, 129.2, 125.9, 66.7, 60.1, 55.3, 27.0, 21.8; MS *m/z* (ESI, negative polarity): MS+1: 431.0; MS2(431.0): 193.7, 188.8, 137.8; MS3(188.8): 137.8, 131.8. Anal. calcd. for C<sub>19</sub>H<sub>20</sub>N<sub>4</sub>O<sub>6</sub>S: C, 52.77; H, 4.66; N, 12.96. Found: C, 52.91; H, 4.70; N, 12.61.

**(3R)-3-Methyl-1-[(1S)-1-(N-methylcarbamoyl)-1-phenyl-methyl]-3-(2-**

**nitrobenzenesulfonylamino)-azetidin-2-one (12e):** According to the aforementioned procedure (compound **12d**) product **12e** was prepared starting from β-lactam (**8e**) (0.31 mmol, 0.130 g) and methylamine (2M in THF; 0.465 mmol, 0.233 mL). An epimerization took place, and the mixture of diastereoisomers **12e** and **12d** (ratio 96:4) was separated by preparative TLC (hexanes/EtOAc, 1:5). Yield: 0.113 g (85%); yellowish powder; m.p. 148–150°C; [α]<sub>D</sub><sup>25</sup> +38.9 (0.7, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr) ν<sub>max</sub>

3210, 2943, 1745, 1725, 1544, 1164, 821  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{H}}$  7.92–7.35 (m, 10H), 5.99 (br s, 1H), 5.93 (br s, 1H), 5.38 (s, 1H), 3.62 (s, 2H), 2.79 (d, 3H,  $J = 4.7$  Hz), 1.62 (s, 3H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{C}}$  168.7, 167.1, 147.5, 135.3, 133.9, 133.5, 132.9, 130.7, 129.2, 128.9, 128.4, 125.3, 65.9, 59.2, 54.8, 26.4, 21.3. MS  $m/z$  (ESI, negative polarity): MS-1: 431.0; MS2(431.0): 137.8, 188.8, 185.7. Anal. calcd. for  $\text{C}_{19}\text{H}_{20}\text{N}_4\text{O}_6\text{S}$ : C, 52.77; H, 4.66; N, 12.96. Found: C, 53.00; H, 4.59; N, 12.58.

**(3R)-3-Methyl-1-[1-(N-methylcarbamoyl)-isopropyl]-3-(2-nitrobenzenesulfonylamino)azetidin-2-one (12f):** The  $\beta$ -lactam (**8f**) (0.162 mmol, 0.160 g) was dissolved in  $\text{CH}_2\text{Cl}_2$  (2.5 mL) and cooled to 0  $^\circ\text{C}$ . To this solution methylamine (2 M THF) (0.323 mmol, 0.162 mL) was added and then the reaction mixture was cooled to  $-12^\circ\text{C}$  (ice/ $\text{NaCl}$ ) and EEDQ (0.072g, 0.292 mmol) was added as a  $\text{CH}_2\text{Cl}_2$  solution (2.5 mL). The reaction was slowly warmed to room temperature and stirred overnight. After this time, the reaction mixture was concentrated under reduced pressure and the crude product was purified by preparative TLC (hexanes/ $\text{EtOAc}$ , 1:7) to afford pure *N*-methylamide **12f** as a white powder. Yield: 0.050 g (75%); m.p.  $135^\circ\text{C}$ ;  $[\alpha]_{\text{D}}^{25} -46.2$  (1.75,  $\text{CH}_2\text{Cl}_2$ ); IR (KBr)  $\nu_{\text{max}}$  3315, 2934, 1758, 1728, 1540, 1166, 798  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta_{\text{H}}$  8.14–7.84 (m, 4H), 3.63 (d, 1H,  $J = 5.0$  Hz), 3.41 (d, 1H,  $J = 5.0$  Hz), 2.79 (s, 3H), 1.57 (s, 3H), 1.54 (s, 3H), 1.45 (s, 3H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ )  $\delta_{\text{C}}$  174.5, 168.3, 148.2, 135.4, 134.1, 132.6, 130.2, 124.8, 64.8, 60.2, 53.8, 25.8, 23.4, 23.3, 20.1. MS  $m/z$  (ESI, negative polarity): MS-1: 383.0; MS2(383.0): 200.7, 185.7, 137.8; MS3(137.8): 117.9. Anal. calcd. for  $\text{C}_{15}\text{H}_{20}\text{N}_4\text{O}_6\text{S}$ : C, 46.87; H, 5.24; N, 14.57. Found: C, 47.02; H, 5.33; N, 14.36.

**General Procedure for the Preparation 3-Acetamido-3-methyl-1-[(N-methylcarbamoyl)-alkyl]-azetidin-2-ones (5a-f):** To a stirred solution of the corresponding  $\beta$ -lactam **12** (0.067 mmol, 0.045 g) in dry  $\text{CH}_3\text{CN}$  (5 mL) thiophenol (0.335 mmol, 0.034 g) and  $\text{K}_2\text{CO}_3$  (0.268 mmol, 0.037 g) were added. After stirring at room temperature for 1 h (monitored by TLC and  $^1\text{H}$  NMR) acetic anhydride (0.402 mmol, 0.088 g) was added and reaction was stirred overnight. The solvent was evaporated under

reduced pressure and crude was purified by preparative TLC (hexanes/EtOAc, 1:7).

**(R)-3-Acetamido-3-methyl-1-[1-(N-methylcarbamoyl)methyl]azetidin-2-one (5a):** The general procedure was followed from  $\alpha$ -(2-nosyl)- $\beta$ -lactam **12a** (0.084 mmol, 0.030 g). The crude product was purified by preparative TLC (hexanes/EtOAc, 1:7). Yield: 0.015 g (84%); yellowish oil;  $[\alpha]_D^{25} +94.8$  (0.5, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr)  $\nu_{\max}$  3210, 2965, 1740, 1728 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta_H$  8.19 (br s, 1H), 6.16 (s, 1H), 4.44 (d, 1H,  $J = 17.6$  Hz), 3.89 (d, 1H,  $J = 5.0$  Hz), 3.57 (d, 1H,  $J = 17.6$  Hz), 3.37 (d, 1H,  $J = 5.0$  Hz), 2.90 (d, 3H,  $J = 4.6$  Hz), 2.06 (s, 3H), 1.58 (s, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta_C$  170.7, 168.8, 168.0, 65.0, 53.5, 45.3, 26.3, 22.9, 20.5. MS m/z (ESI, negative polarity): MS-1: 211.8; MS2(212.0): 181.8, 169.8, MS3(169.8): 126.8, 112.8, 100.8, 96.0, 82.9. Anal. calcd. for C<sub>9</sub>H<sub>15</sub>N<sub>3</sub>O<sub>3</sub>: C, 50.69; H, 7.09; N, 19.71. Found: C, 50.72; H, 7.35; N, 19.62.

**(3R)-3-Acetamido-3-methyl-1-[1-(1R)-(N-methylcarbamoyl)ethyl]azetidin-2-one (5b):** The general procedure was followed from  $\alpha$ -(2-nosyl)- $\beta$ -lactam **12b** (0.064 mmol, 0.024 g). The crude product was purified by preparative TLC (hexanes/EtOAc, 1:7). Yield: 0.010 g (72%); yellowish solid; m.p. 159-160°C;  $[\alpha]_D^{25} +58.2$  (0.5, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr)  $\nu_{\max}$  3125, 3060, 1782, 1768 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta_H$  8.13 (br s, 1H), 5.96 (s, 1H), 4.47 (q, 1H,  $J = 7.3$  Hz), 3.73 (d, 1H,  $J = 5.1$  Hz), 3.23 (d, 1H,  $J = 5.1$  Hz), 2.84 (d, 3H,  $J = 4.6$  Hz), 2.04 (s, 3H), 1.55 (s, 3H), 1.40 (d, 3H,  $J = 7.3$  Hz); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta_C$  170.6, 168.6, 63.5, 50.2, 49.8, 26.5, 23.0, 20.7, 14.4. MS m/z (ESI, negative polarity): MS-1: 225.9; MS2(225.9): 183.8; MS3(183.8): 126.8, 96.9. Anal. calcd. for C<sub>10</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub>: C, 52.85; H, 7.54; N, 18.49. Found: C, 52.86; H, 7.60; N, 18.53.

**(3R)-3-Acetamido-3-methyl-1-[1-(1R)-(N-methylcarbamoyl)ethyl]azetidin-2-one (5c):** The general procedure was followed from  $\alpha$ -(2-nosyl)- $\beta$ -lactam **12c** (0.060 mmol, 0.022 g). The crude product was purified by preparative TLC (hexanes/EtOAc, 1:7). Yield: 0.011 g (81%); white powder; m.p. 115°C;  $[\alpha]_D^{25} +58.6$  (0.5, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr)  $\nu_{\max}$  3310, 3140, 1785, 1758 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta_H$  8.01 (br s, 1H), 5.96 (s, 1H), 3.80 (d, 1H,  $J = 5.0$  Hz), 3.75 (q, 1H,  $J = 7.4$  Hz), 3.17 (d, 1H,  $J = 5.0$  Hz), 2.86 (d, 3H,  $J = 4.7$  Hz), 2.03 (s, 3H), 1.73 (d, 3H,  $J = 7.5$  Hz), 1.55 (s, 3H); <sup>13</sup>C NMR

(125 MHz, CDCl<sub>3</sub>)  $\delta_C$  171.4, 170.5, 168.6, 63.4, 56.2, 53.5, 26.4, 23.0, 20.6, 16.3. MS m/z (Ion Source Type: ESI, negative polarity): MS-1: 225.9; MS2(225.9): 195.8, 183.8; MS3(183.8): 126.8, 96.9. Anal. calcd. for C<sub>10</sub>H<sub>17</sub>N<sub>3</sub>O<sub>3</sub>: C, 52.85; H, 7.54; N, 18.49. Found: C, 52.59; H, 7.68; N, 18.84.

**(3R)-3-Acetamido-3-methyl-1-[1-(1R)-(N-methylcarbamoyl)phenylmethyl]azetidin-2-one (5d):**

The general procedure was followed from  $\alpha$ -(2-nosyl)- $\beta$ -lactam **12d** (0.108 mmol, 0.047 g). The crude product was purified by preparative TLC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 10:1). Yield: 0.028 g (89%); white powder; m.p. 99°C;  $[\alpha]_D^{25}$  +21.2 (0.5, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr)  $\nu_{\max}$  3125, 3094, 1760, 1725 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta_H$  8.44 (br s, 1H), 7.37-7.22 (m, 5H), 5.99 (br s, 1H), 5.50 (s, 1H), 3.73 (d, 1H, *J* = 5.0 Hz), 2.95 (d, 3H, *J* = 4.6 Hz), 2.72 (d, 1H, *J* = 5.0 Hz), 2.00 (s, 3H), 1.38 (s, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta_C$  170.9, 169.2, 168.3, 134.3, 129.0, 128.9, 128.4, 63.4, 59.4, 51.3, 26.6, 22.9, 20.2. MS m/z (Ion Source Type: ESI, negative polarity): MS-1: 288.1; MS2(288.0): 245.8, 230.8, 171.8, 126.8, 111.9; MS3(245.8): 126.8; MS4(126.8): 111.8, 98.9. Anal. calcd. for C<sub>15</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>: C, 62.27; H, 6.62; N, 14.52. Found: C, 62.40; H, 6.51; N, 14.75.

**(3R)-3-Acetamido-3-methyl-1-[1-(1S)-(N-methylcarbamoyl)phenylmethyl]azetidin-2-one (5e):**

The general procedure was followed from  $\alpha$ -(2-nosyl)- $\beta$ -lactam **12e** (0.144 mmol, 0.062 g). The crude product was purified by preparative TLC (CH<sub>2</sub>Cl<sub>2</sub>/MeOH 10:1). Yield: 0.039 g (94%); white crystals; m.p. 198-199°C;  $[\alpha]_D^{25}$  +133.5 (0.75, MeOH); IR (KBr)  $\nu_{\max}$  3210, 2998, 1744, 1725 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta_H$  7.43-7.39 (m, 5H), 7.14 (br s, 1H), 5.66 (s, 1H), 5.77 (s, 1H), 3.77 (d, 1H, *J* = 5.0 Hz), 3.46 (d, 1H, *J* = 5.0 Hz), 2.93 (d, 3H, *J* = 4.7 Hz), 2.02 (s, 3H), 1.58 (s, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta_C$  170.1, 168.9, 168.3, 134.6, 128.8, 128.7, 128.5, 76.6, 63.4, 62.3, 53.9, 26.5, 23.0, 20.4. MS m/z (Ion Source Type: ESI, negative polarity): MS-1: 288.1; MS2(288.0): 245.8, 230.8, 188.8, 171.8, 126.8; MS3(245.8): 126.8; MS4(126.8): 111.8, 58.3. Anal. calcd. for C<sub>15</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>: C, 62.27; H, 6.62; N, 14.52. Found: C, 62.67; H, 6.70; N, 14.30.

**(3R)-3-Acetamido-3-methyl-1-[1-(N-methylcarbamoyl)isopropyl]azetidin-2-one (5f):** The general procedure was followed from  $\alpha$ -(2-nosyl)- $\beta$ -lactam **12f** (0.091 mmol, 0.035 g). The crude product was

purified by preparative TLC (hexanes/EtOAc, 1:7). Yield: 0.018 g (82%); yellowish powder; m.p. 216-218°C [ $\alpha$ ]<sub>D</sub><sup>25</sup> +57.7 (0.75, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr)  $\nu_{\text{max}}$  3320, 3180, 1769, 1748 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta_{\text{H}}$  8.28 (br s, 1H), 6.30 (s, 1H), 3.73 (d, 1H,  $J$  = 5.0 Hz), 3.20 (d, 1H,  $J$  = 5.0 Hz), 2.82 (d, 3H,  $J$  = 4.6 Hz), 2.02 (s, 3H), 1.71 (s, 3H), 1.52 (s, 3H), 1.36 (s, 3H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta_{\text{C}}$  173.7, 170.7, 168.3, 62.2, 60.3, 51.0, 26.6, 24.8, 24.3, 22.9, 20.7. MS  $m/z$  (Ion Source Type: ESI, positive polarity): MS+23: 264.0, 242.0; MS2(242.0): 129.0; MS3(129.0): 101.1, 58.4. Anal. calcd. for C<sub>11</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>: C, 54.76; H, 7.94; N, 17.42. Found: C, 54.50; H, 8.00; N, 17.09.

**General Procedure for the Preparation of Ns-( $\beta$ -Lactam)-Aa-Aib-OBn Compounds (13b-e):** To a stirred solution of the corresponding *N*-carboxymethyl  $\beta$ -lactam **9b-e** (1 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) cooled to -0°C was added H-Aib-OBn (1 mmol, 0.193 g) dissolved in CH<sub>2</sub>Cl<sub>2</sub> (9 mL). The solution was cooled to -12 °C, EEDQ (1.2 mmol, 0.310 g) was added and the reaction mixture was slowly warmed to room temperature and stirred overnight. The resulting solution was washed with 1M HCl (3x10 mL), the aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> and the combined organic phases were dried (MgSO<sub>4</sub>) and evaporated. The product was purified by flash column chromatography (hexanes/EtOAc, 2:1).

**Compound 13b:** The general procedure was followed from the *N*-carboxyalkyl- $\beta$ -lactam **9b** (1 mmol, 0.433 g); Yield: 0.486 g (79%); white solid; m.p. 216-218°C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +54.7 (0.42, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr)  $\nu_{\text{max}}$  3396, 3085, 1762, 1681, 1539, 1347, 1153 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta_{\text{H}}$  8.15-7.72 (m, 4H, o-Ns), 7.37-7.21 (m, 10H, Ar), 7.17 (s, 1H, NHCMe<sub>2</sub>), 5.89 (s, 1H, NH-Ns), 5.21 (d, 1H,  $J$  = 12.3 Hz, OCH<sub>2</sub>Ph), 5.09 (d, 1H,  $J$  = 12.6 Hz, OCH<sub>2</sub>Ph), 4.32 (m, 1H, NCHMe), 3.55 (d, 1H,  $J$  = 5.7 Hz, CH<sub>2</sub>NCO), 3.34 (d, 1H,  $J$  = 5.7 Hz, CH<sub>2</sub>NCO), 3.14 (d, 1H,  $J$  = 13.5 Hz, NCCH<sub>2</sub>Ph), 3.03 (d, 1H,  $J$  = 13.5 Hz, NCCH<sub>2</sub>Ph), 1.59 (s, 3H, Me<sub>2</sub>C), 1.56 (s, 3H, Me<sub>2</sub>C), 0.83 (d, 3H,  $J$  = 6.9 Hz, MeCHNH); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta_{\text{C}}$  173.8, 168.8, 166.1, 147.4, 134.9, 133.8, 133.2, 132.6, 130.9, 130.2, 128.9, 128.5, 128.2, 128.1, 125.3, 70.1, 67.0, 56.6, 50.5, 49.5, 40.3, 25.4, 24.7, 13.4. MS  $m/z$  (Ion Source Type: ESI, positive polarity): MS+23: 264.0, 242.0; MS2(242.0): 129.0; MS3(129.0): 101.1, 58.4. Anal.

calcd. for  $C_{30}H_{32}N_4O_8S$ : C, 59.20; H, 5.30; N, 9.20. Found: C, 58.86; H, 5.64; N, 9.01.

**Compound 13c:** The general procedure was followed from the *N*-carboxyalkyl- $\beta$ -lactam **9b** (1 mmol, 0.433 g). Yield: 0.426 g (70%); white solid; m.p. 216–218°C;  $[\alpha]_D^{25} +106.7$  (0.3,  $CH_2Cl_2$ ); IR (KBr)  $\nu_{max}$  3378, 3105, 1748, 1539, 1356, 1165  $cm^{-1}$ .  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta_H$  8.14–7.74 (m, 4H, o-Ns), 7.40–7.25 (m, 10H, Ar), 6.93 (s, 1H,  $NHCMe_2$ ), 5.86 (s, 1H,  $NH$ -Ns), 5.18 (s, 2H,  $OCH_2Ph$ ), 3.89 (q, 1H,  $J = 1.6$  Hz,  $NCH(Me)CO$ ), 3.72 (d, 1H,  $J = 5.7$  Hz,  $CH_2NCO$ ), 3.47 (d, 1H,  $J = 5.7$  Hz,  $CH_2NCO$ ), 3.14 (q, 2H,  $J = 1.6$  Hz,  $CqCH_2Ph$ ), 1.59 (s, 3H,  $Me_2C$ ), 1.58 (s, 3H,  $Me_2C$ ), 1.46 (d, 3H,  $J = 7.25$  Hz,  $MeCHNH$ );  $^{13}C$  NMR (125 MHz,  $CDCl_3$ )  $\delta_C$  173.9, 169.5, 166.5, 147.4, 133.6, 133.2, 133.1, 130.9, 130.0, 129.0, 128.5, 128.2, 128.1, 127.9, 125.3, 69.5, 67.2, 56.6, 53.8, 53.5, 40.6, 29.7, 24.9, 15.1. MS  $m/z$  (TOF MS ES+): MS[+Na]: 630.9, MS[+H]: 608.8, MS: 562.8, 500.9, 472.9, 394.0, 359.9, 115.9. Anal. calcd. for  $C_{30}H_{32}N_4O_8S$ : C, 59.20; H, 5.30; N, 9.20. Found: C, 59.14; H, 5.55; N, 8.92.

**Compound 13d:** The general procedure was followed from the *N*-carboxyalkyl- $\beta$ -lactam **9d** (1 mmol, 0.497 g). Yield: 0.368 g (55%); white solid; m.p. 72–74°C;  $[\alpha]_D^{25} -83.8$  (0.65,  $CH_2Cl_2$ ); IR (KBr)  $\nu_{max}$  3378, 3028, 1768, 1742, 1677, 1534, 1148  $cm^{-1}$ ;  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta_H$  8.15–7.68 (m, 4H), 7.50–6.85 (m, 15H), 5.90 (br s, 1H), 5.32 (s, 1H), 5.20 (d, 1H,  $J = 12.5$  Hz), 5.16 (d, 1H,  $J = 12.5$  Hz), 3.69 (d, 1H,  $J = 6.0$  Hz), 3.04 (d, 1H,  $J = 6.0$  Hz), 3.00 (d, 1H,  $J = 14.0$  Hz), 2.95 (d, 1H,  $J = 14.0$  Hz), 1.67 (s, 3H), 1.64 (s, 3H);  $^{13}C$  NMR (125 MHz,  $CDCl_3$ )  $\delta_C$  173.8, 167.8, 165.9, 147.5, 136.0, 134.8, 134.0, 133.4, 133.3, 132.5, 131.1, 130.1, 129.1, 128.9, 128.7, 128.6, 128.5, 128.2, 128.1, 128.0, 125.5, 69.9, 67.2, 59.2, 55.9, 52.3, 40.3, 25.5, 24.9. MS  $m/z$  (Ion Source Type: ESI, positive polarity): MS: 693.2; MS2(693.2): 671.2, 666.1, 665.2, 478.3; MS3(665.2): 479.2, 478.2, 463.1. Anal. calcd. for  $C_{35}H_{34}N_4O_8S$ : C, 62.67; H, 5.11; N, 8.35. Found: C, 62.60; H, 4.95; N, 8.07.

**Compound 13e:** The general was followed from the *N*-carboxyalkyl- $\beta$ -lactam **9e** (0.42 mmol, 0.2 g) and  $\alpha$ -aminoisobutyric acid benzyl ester (0.44 mmol; 0.085 g). Yield: 0.023 g (66%); m.p. 64–65°C;  $[\alpha]_D^{25} +9.4$  (0.65,  $CH_2Cl_2$ ); IR (KBr)  $\nu_{max}$  3350, 2928, 1744, 1726, 1682, 1536, 1148  $cm^{-1}$ ;  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta_H$  7.95–7.61 (m, 4H); 7.37–7.26 (m, 15H), 6.44 (br s, 1H), 5.81 (br s, 1H), 5.33 (s,

1H), 5.19 (d, 1H,  $J = 12.5$  Hz), 5.15 (d, 1H,  $J = 12.5$  Hz), 3.76 (d, 1H,  $J = 5.5$  Hz), 3.52 (d, 1H,  $J = 5.5$  Hz), 3.25 (dd, 2H,  $J = 11.9$  Hz), 1.59 (s, 3H), 1.56 (s, 3H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ )  $\delta_{\text{C}}$  173.9, 167.4, 166.7, 147.3, 135.6, 135.3, 133.6, 133.5, 133.0, 131.1, 129.9, 129.2, 129.1, 129.0, 128.7, 128.5, 128.2, 127.9, 125.4, 69.3, 67.5, 59.7, 57.1, 53.6, 41.1, 25.0, 24.5. MS  $m/z$  (Ion Source Type: ESI, positive polarity): MS: 693.3; MS2(693.2): 666.1, 665.2, 478.2, 361.2; MS3(665.2): 479.1, 478.2, 463.2. Anal. calcd. for  $\text{C}_{35}\text{H}_{34}\text{N}_4\text{O}_8\text{S}$ : C, 62.67; H, 5.11; N, 8.35. Found C, 62.32; H, 5.06; N, 7.98.

### General Procedure for the Preparation of Boc-( $\beta$ -Lactam)-Aa-Aib-OH Compounds **6b-e**:

Thiophenol (5.00 mmol, 0.469 mL) and  $\text{K}_2\text{CO}_3$  (4.00 mmol, 0.567 g) were added to a solution of the corresponding Ns-( $\beta$ -Lactam)-Aa-Aib-OBn **13b-e** (1 mmol) in dry MeCN (15 mL) and the suspension was stirred at room temperature for 2 h (monitored by TLC and  $^1\text{H}$  NMR). Upon completion, the reaction mixture was filtered through a pad of celite, evaporated in vacuo and quickly purified by column chromatography ( $\text{CH}_2\text{Cl}_2$ , then MeOH). The corresponding intermediate amine H-( $\beta$ -Lactam)-Aa-Aib-OBn was redissolved in MeCN (15 mL), di-*tert*-butyldicarbonate (0.402 mmol; 0.088 g) was added and the solution was stirred overnight at room temperature. The solution was evaporated, treated with saturated aqueous  $\text{NaHCO}_3$  and extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 10 mL). The combined organic solutions were dried ( $\text{MgSO}_4$ ) and the solvents were evaporated under reduced pressure to afford the corresponding intermediate Boc-( $\beta$ -Lactam)-Aa-Aib-OBn, which was purified by preparative TLC (hexanes/EtOAc, 2:1). This benzyl ester was dissolved in ethanol (15 mL) containing 10%Pd/C (0.050 g), and the suspension was stirred at room temperature under hydrogen for 16 h. After this time, the reaction mixture was filtered through a pad of celite, washed with ethanol (2 x 10 mL), and the combined organic solution was evaporated under reduced pressure to afford the corresponding carboxyl peptide product **6b-e**.

**2-{2-[(3*R*)-3-Benzyl-3-(*tert*-butoxycarbonylamino)-2-oxoazetidin-1-yl]-(*R*)-propanamido}-2-methylpropanoic acid (**6b**)** : The general procedure was followed from compound **13b** (1 mmol, 0.608



g). Overall yield: 0.342 g (79%); Oil;  $[\alpha]_D^{25} -15.0$  (0.2, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr)  $\nu_{\max}$  3320, 3180, 1769, 1748 cm<sup>-1</sup>. <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD)  $\delta_H$  7.31-7.29 (m, 5H, Ar), 4.0 (m, 1H, CHMe), 3.67 (d, 1H,  $J = 4.8$  Hz, CH<sub>2</sub>NCO), 3.35 (d, 1H,  $J = 4.8$  Hz, CH<sub>2</sub>NCO), 3.07 (d, 1H,  $J = 13.3$  Hz, CH<sub>2</sub>Ph), 2.97 (d, 1H,  $J = 13.3$  Hz, CH<sub>2</sub>Ph), 1.47 (s, 9H, *t*BuO), 1.46 (s, 6H, Me<sub>2</sub>CH), 0.58 (d, 3H,  $J = 7.1$  Hz, MeCH); <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD)  $\delta_C$  181.2, 171.2, 171.0, 156.6, 135.6, 131.8, 129.4, 128.4, 81.0, 68.8, 59.5, 50.8, 39.1, 28.7, 26.9, 25.8, 13.9. HPLC-MS, MeOH/HCO<sub>2</sub>H *m/z* (ESI, positive polarity): MS+23: 264.0, 242.0; MS2(242.0): 129.0; MS3(129.0): 101.1, 58.4. Anal. calcd. for C<sub>22</sub>H<sub>31</sub>N<sub>3</sub>O<sub>6</sub>: C, 60.95; H, 7.21; N, 9.69. Found C, 61.33; H, 6.95; N, 9.60.

**2-{2-[(3*R*)-3-Benzyl-3-(*tert*-butoxycarbonylamino)-2-oxoazetidin-1-yl]-(*S*)-propanamido}-2-**

**methylpropanoic acid (6c)** : The general procedure was followed from compound **13c** (1 mmol, 0.608 g). Overall yield: 0.346 g (80%); white solid; m.p. 216-218°C;  $[\alpha]_D^{25} -28.1$  (0.36, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr)  $\nu_{\max}$  2974, 1739, 1698, 1583, 1363, 1166 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta_H$  7.77 (s, 1H, NHCM<sub>2</sub>), 7.39-7.19 (m, 5H, Ar), 4.9 (s, 1H, BocHN), 4.34 (m, 1H, CHMe), 3.65 (d, 1H,  $J = 4.4$  Hz, CH<sub>2</sub>NCO), 3.38 (d, 1H,  $J = 5.4$  Hz, CH<sub>2</sub>NCO), 3.19 (d, 1H,  $J = 13.5$  Hz, CH<sub>2</sub>Ph), 2.94 (d, 1H,  $J = 13.5$  Hz, CH<sub>2</sub>Ph), 1.55 (s, 3H, Me<sub>2</sub>C), 1.55 (s, 3H, Me<sub>2</sub>C), 1.45 (s, 9H, *t*BuO), 0.85 (d, 3H,  $J = 7.25$  Hz, MeCH); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta_C$  180.6, 170.2, 169.8, 155.5, 134.6, 130.7, 128.4, 127.4, 79.9, 67.7, 58.4, 49.8, 38.1, 29.7, 27.7, 25.8, 24.8, 12.9. HRMS (TOF CI) *m/z*: 433.2221; C<sub>22</sub>H<sub>31</sub>N<sub>3</sub>O<sub>6</sub> requires: 433.2216.

**2-[[3(*R*)-3-Benzyl-3-(*tert*-butoxycarbonylamino)-2-oxoazetidin-1-yl]-(*R*)-phenylacetamido]-2-**

**methylpropanoic acid (6d)** : The general procedure was applied from β-lactam **13d** (1.00 mmol, 0.670 g). Yield: 0.355 g (82%); white solid; mp = 238 °C;  $[\alpha]_D^{25} +35.8$  (0.65, MeOH); IR (KBr)  $\nu_{\max}$  3320, 2928, 2851, 1744, 1686, 1670, 1651 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD)  $\delta_H$  7.32-6.64 (m, 10H), 5.03 (s, 1H), 3.58 (d, 1H,  $J = 5.5$  Hz), 3.02 (d, 1H,  $J = 13.5$  Hz), 2.85 (d, 1H,  $J = 13.5$  Hz), 2.83 (d, 1H,  $J = 5.5$  Hz), 1.61 (s, 3H), 1.57 (s, 3H), 1.50 (s, 9H); <sup>13</sup>C NMR (125 MHz, CD<sub>3</sub>OD)  $\delta_C$  176.8, 169.7, 168.9, 155.4, 133.7, 133.6, 130.3, 128.7, 128.3, 127.8, 127.2, 79.8, 67.6, 58.6, 56.8, 48.5, 27.3, 25.4, 23.7. HPLC-MS, MeOH/HCO<sub>2</sub>H *m/z* (ESI, positive polarity): 566.4; MS2(566.4): 436.1, 435.0, 434.2, 374.1,

202.1; MS3(434.2): 375.1, 374.1, 188.0, 187.0, 178.0, 173.0, 172.0, 159.0, 131.0, 130.0; MS4(374.1): 188.0, 187.0, 172.0, 170.0, 159.0, 130.9. Anal. calcd. for C<sub>27</sub>H<sub>33</sub>N<sub>3</sub>O<sub>6</sub>: C, 65.44; H, 6.71; N, 8.48. Found C, 65.30; H, 6.49; N, 8.13.

**2-[[*(3R)*-3-Benzyl-3-(*tert*-butoxycarbonylamino)-2-oxoazetidin-1-yl]-(*S*)-phenylacetamido]-2-methylpropanoic acid (6e):** The general procedure was applied from  $\beta$ -lactam **13e** (1.00 mmol, 0.670 g). Yield: 0.346 g (80%); mp 113–115 °C;  $[\alpha]_D^{25} +44.5$  (0.3, MeOH); IR (KBr)  $\nu_{\max}$  3326, 2980, 2916, 2853, 1744, 1698, 1674, 1660 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta_H$  8.41 (s, 1H), 7.30–7.22 (m, 10H), 5.29 (s, 1H), 3.48 (br s, 1H), 3.32 (br s, 1H), 3.17–3.10 (br s, 2H), 1.36 (br s, 15H). <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  176.8, 168.1, 167.3, 154.8, 136.3, 136.1, 130.5, 128.7, 128.5, 128.4, 128.1, 127.0, 79.2, 67.5, 58.4, 56.0, 51.4, 38.8, 28.6, 25.2, 25.0. HPLC-MS *m/z* (ESI, negative polarity): 494.3; MS2(494.3): 420.0; MS3(420.0): 377.0, 334.0, 333.1, 291.0, 273.0, 260.9, 249.9, 248.0, 174.8, 127.9; MS4(377.0): 333.0, 275.9, 249.9, 247.9, 207.0, 159.9, 127.9 188.0, 187.0, 172.0, 170.0, 159.0, 130.9. Anal. calcd. for C<sub>27</sub>H<sub>33</sub>N<sub>3</sub>O<sub>6</sub>: C, 65.44; H, 6.71; N, 8.48. Found C, 65.14; H, 6.98; N, 8.20.

**Preparation of 2-[[*(3R)*-3-Benzyl-3-(*tert*-butoxycarbonylamino)-2-oxoazetidin-1-yl]-dimethylacetamido]-2-methylpropanoic acid (6f).**

**(3R)-3-Benzyl-3-*tert*-butoxycarbonyl-1-[1-(methoxycarbonyl)isopropyl]-azetidin-2-one.**

Thiophenol (5 mmol, 0.469 mL) and K<sub>2</sub>CO<sub>3</sub> (4 mmol, 0.567 g) were added to a solution of (*3R*)-3-benzyl-3-(2-nitrobenzenesulfonylamino)-1-[1-(methoxycarbonyl)isopropyl]-azetidin-2-one<sup>1</sup> (1 mmol, 0.461 g) in MeCN (5 mL). The suspension was stirred for 2 h at r.t., the solvent was evaporated in vacuo and the residue was purified by column chromatography (EtOAc, then MeOH) to afford (*3R*)-3-benzyl-3-amino-1-[1-(methoxycarbonyl)isopropyl]-azetidin-2-one in 90% yield (0.248 g). To a solution of this material in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added (Boc)<sub>2</sub>O (3 mmol, 0.654g) and the mixture was stirred at r.t. for 16 h. After evaporation of the solvent, the crude was purified by column chromatography hexanes/EtOAc, 5:1). Overall yield: 0.188 g (50%); Yellow oil;  $[\alpha]_D^{25} +10.2$  (0.6, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr)  $\nu_{\max}$  3318, 1760, 1742, 1714 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta_H$  7.38-7.25 (m, 5H, Ph), 4.78 (s, 1H,

NH), 3.66 (s, 3H, OCH<sub>3</sub>), 3.58 (d, 1H, *J* = 5.2 Hz, CH<sub>2</sub>NCO), 3.38 (d, 1H, *J* = 5.2 Hz, CH<sub>2</sub>NCO), 3.11 (s, 2H, CH CH<sub>2</sub>Ph), 1.48 (s, 9H, *t*BuO), 1.35 (s, 3H, CMe<sub>2</sub>), 1.21 (s, 3H, CMe<sub>2</sub>). <sup>13</sup>C NMR (75MHz, CDCl<sub>3</sub>): 173.2, 166.9, 153.4, 135.3, 130.3, 128.4, 127.1, 65.8, 58.8, 52.6, 49.9, 39.1, 28.2, 23.7, 23.6. MS *m/z* (ESI, positive polarity): +MS2: 343.1, +MS4: 283.0. Anal. calcd. for C<sub>20</sub>H<sub>28</sub>N<sub>2</sub>O<sub>5</sub>: C, 63.81; H, 7.50; N, 7.44. Found: C, 63.64; H, 7.36; N, 7.19.

**Compound (6f):** To a solution of (3*R*)-3-benzyl-3-*tert*-butoxycarbonyl-1-[1-(methoxycarbonyl)isopropyl]-azetidin-2-one (1 mmol, 0.376 g) in MeOH (20 mL) was added LiOH.H<sub>2</sub>O (10 mmol, 0.485 g) in 10 mL of H<sub>2</sub>O and the mixture was stirred at r.t. for 4h. Acidification of the resulting mixture with 1M HCl and extraction with CH<sub>2</sub>Cl<sub>2</sub> (3 x 8 mL) provided an organic solution of (3*R*)-3-benzyl-3-*tert*-butoxycarbonyl-1-[1-(carboxy)isopropyl]-azetidin-2-one, which was dried (Na<sub>2</sub>SO<sub>4</sub>) and filtered. H-Aib-OBn (1.3 mmol, 0.248 g) was added, the mixture was cooled to -12°C and EEDQ (1.3 mmol, 0.331 g) was added. The reaction mixture was stirred during 16 h, while reaching r.t. slowly. The resulting solution was washed with 1M HCl (3x10 mL), the aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> and the combined organic phases were dried (MgSO<sub>4</sub>) and evaporated. The crude was purified by flash column chromatography (hexanes/EtOAc, 5:1) affording benzyl 2-[(3*R*)-3-benzyl-3-(*tert*-butoxycarbonylamino)-2-oxoazetidin-1-yl]-dimethylacetamido}-2-methylpropanoate; white solid; 0.494 g (92%). A suspension of this benzyl ester (0.92 mmol) and 20%-Pd/C in EtOAc (35 mL) was stirred for 1h at r.t. in H<sub>2</sub> atmosphere. The mixture was filtered through a celite pad and the filtrate was evaporated. Yield: 0.411 g (100%); white solid; m.p. 213-215°C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +65.2 (0.48, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr): 3346, 1736, 1702, 1685 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ <sub>H</sub> 7.87 (s, 1H, NH<sub>2</sub>CMe<sub>2</sub>), 7.38-7.27 (m, 5H, Ar), 4.84 (s, 1H, BocNH), 3.62 (d, 1H, *J* = 5.1 Hz, CH<sub>2</sub>NCO), 3.35 (d, 1H, *J* = 5.1 Hz, CH<sub>2</sub>NCO), 3.14 (d, 1H, *J* = 13.4 Hz, CH<sub>2</sub>Ph), 2.89 (d, 1H, *J* = 13.4 Hz, CH<sub>2</sub>Ph), 1.58 (s, 3H, Me<sub>2</sub>C), 1.57 (s, 3H, Me<sub>2</sub>C), 1.45 (s, 9H, *t*BuO), 1.32 (s, 3H, Me<sub>2</sub>C), 0.95 (s, 3H, Me<sub>2</sub>C). <sup>13</sup>C NMR (75MHz, CDCl<sub>3</sub>): 175.8, 174.5, 168.3, 154.7, 133.5, 130.8, 129.1, 128.2, 81.6, 66.4, 59.9, 57.9, 48.6, 39.5, 28.7, 25.9, 25.3, 24.4, 23.8. MS *m/z* (ESI, positive polarity): MS: 446.2, MS2(446.2): 372.8, 372.1, MS3(372.1): 329.0,

MS4(329.0): 329.1, 242.9, 201.9, 199.9, 172.0, 168.9, 153.9, 127.9, 111.0. Anal. calcd. for  $C_{23}H_{33}N_3O_6$ : C, 61.73; H, 7.43; N, 9.39. Found: C, 62.04; H, 7.39; N, 8.93.

### General Procedure for the Preparation of Boc-Ala-( $\alpha$ -Bn- $\beta$ -Lactam)-Aib-OMe Compounds 15-

**16:** Thiophenol (5 mmol, 0.469 mL) and  $K_2CO_3$  (4 mmol, 0.567 g) were added to a stirred solution of (3*R*)-3-benzyl-1-[1-(methoxycarbonyl)isopropyl]-3-(2-nitrobenzenesulfonylamino)-azetidin-2-one **14** (1 mmol, 0.461 g) in MeCN (5 mL). The mixture was stirred over 2 h, the solvent was evaporated in vacuo and the crude was purified by flash column chromatography (EtOAc, then MeOH). Yield: 90%. A solution of the resulting amine and the corresponding Boc-Ala-OH (1.3 mmol, 0.248 g) in anhydrous  $CH_2Cl_2$  (20 mL) was cooled to  $-12^\circ C$  and EEDQ (1.3 mmol, 0.331 g) was added. The mixture was stirred during 16 h, while reaching r.t. slowly. The resulting solution was washed with 1M HCl (3x10 mL), the aqueous layer was extracted with  $CH_2Cl_2$  and the combined organic phases were dried ( $MgSO_4$ ) and evaporated. The product was purified by flash column chromatography (hexanes/EtOAc, 2:1).

**Methyl 2-[(3*R*)-3-benzyl-3-[2-(*tert*-butoxycarbonylamino)-(2*R*)-propanamido]-2-oxoazetidin-1-yl]-2-methylpropanoate (15):** The general procedure was followed from compound **17** (1 mmol, 0.461 g) and Boc-D-Ala-OH (1.3 mmol, 0.248 g). Yield: 0.371 g (83%); white solid; m.p.  $135-137^\circ C$ ;  $[\alpha]_D^{25} +28.5$  (0.2,  $CH_2Cl_2$ ); IR (KBr)  $\nu$  3309, 3000, 1763, 1752, 1704, 1656, 1160, 672  $cm^{-1}$ ;  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta_H$  7.28-7.32 (m, 5H, Ar), 6.62 (bs, 1H,  $CONH\text{CCH}_2Ph$ ), 4.9 (bs, 1H,  $CONHCH(CH_3)CO$ ), 4.14 (m, 1H,  $NHCH(Me)CO$ ), 3.71 (s, 3H,  $CO_2Me$ ), 3.63 (d, 1H,  $J = 5.5$  Hz,  $NCH_2CCH_2Ph$ ), 3.45 (d, 1H,  $J = 5.4$  Hz,  $NCH_2CCH_2Ph$ ), 3.21 (d, 1H,  $J = 14.2$  Hz,  $CH_2Ph$ ), 3.18 (d, 1H,  $J = 14.0$  Hz,  $CH_2Ph$ ), 1.45 (s, 9H,  $tBuO$ ), 1.43 (s, 3H,  $Me_2CCO_2Me$ ), 1.36 (s, 3H,  $Me_2CCO_2Me$ ), 1.31 (d, 3H,  $J = 7.1$  Hz,  $CH(Me)CONH$ ).  $^{13}C$ -NMR (75MHz,  $CDCl_3$ ): 173.1, 172.7, 166.8, 155.3, 134.8, 130.2, 128.4, 127.2, 80.2, 65.7, 58.9, 52.5, 49.4, 38.7, 28.3, 23.6, 18.3, 16.8. HPLC-MS, MeOH/ $HCO_2H$  m/z (ESI, positive polarity): 187.1(35), 133.1(99), 126.1(62), 112.1(41), 97.1(41), 72.1(85), 59.1(100),

57.1(48), 55.1(53). HRMS (TOF CI)  $m/z$ : 447.2372;  $C_{23}H_{33}N_3O_6$  requires: 447.2369.

**Methyl 2-[[[(3*R*)-3-benzyl-3-[2-(*tert*-butoxycarbonylamino)-(2*S*)-propanamido]-2-oxoazetidin-1-yl]-2-methylpropanoate (16):** The general procedure was followed from compound **17** (1 mmol, 0.461 g) and Boc-Ala-OH (1.3 mmol, 0.248 g). Yield: 0.380 g (85%); yellowish solid;  $[\alpha]_D^{25}$   $-22.4$  (0.5,  $CH_2Cl_2$ ); IR (KBr)  $\nu$  3271, 2993, 1753, 1721, 1716, 1673, 1167, 740  $cm^{-1}$ ;  $^1H$  NMR (500 MHz,  $CDCl_3$ )  $\delta_H$  7.32 (m, 5H, Ar), 4.08 (m, 1H,  $COCH(Me)NH$ ), 3.65 (s, 3H,  $CO_2Me$ ), 3.59 (d, 1H,  $J = 5.5$  Hz,  $NCH_2C$ ), 3.41 (d, 1H,  $J = 5.3$  Hz,  $NCH_2C$ ), 3.15 (dd, 2H,  $J = 13.7$  Hz,  $CH_2Ph$ ), 1.45 (s, 9H,  $tBuO$ ), 1.33 (s, 3H,  $CMe_2CO_2Me$ ), 1.29 (d, 1H,  $J = 6.9$  Hz,  $CHMeNH$ ), 1.23 (s, 3H,  $CMe_2CO_2Me$ );  $^{13}C$ -NMR (75MHz,  $CDCl_3$ ): 173.2, 172.9, 155.3, 134.9, 130.3, 128.4, 127.1, 79.6, 65.9, 58.9, 53.5, 52.5, 49.8, 38.5, 28.3, 23.4, 18.3. HPLC-MS, MeOH/ $HCO_2H$   $m/z$  (ESI, positive polarity): 187.1(32), 134.1(28), 133.1(100), 115.6(27), 102.5(21), 92.1(33), 91.1(41), 58(56). HRMS (TOF CI)  $m/z$ : 447.2348;  $C_{23}H_{33}N_3O_6$  requires: 447.2369.

#### General Procedure for the Preparation of Boc-Ala-( $\beta$ -Lactam)-Aib-Ala- $NH_2$ Compounds 7a-d:

To a solution of the corresponding methyl 2-[[[(3*R*)-3-benzyl-3-[2-(*tert*butoxycarbonylamino)-2*R*-propanamido]-2-oxoazetidin-1-yl]-2-dimethylpropanoate **15** or **16** (1 mmol, 0.447 g) in MeOH (20 mL) was added a solution of  $LiOH \cdot H_2O$  (10 mmol, 0.485 g) in MeOH/ $H_2O$  10:5 mL. The mixture was stirred for 2 h, it was acidified with 1M HCl (5 mL) and the aqueous phase was extracted with  $CH_2Cl_2$  (3 x 15 mL) to afford the intermediate carboxylic acid in 85% yield. To this crude material dissolved in anhydrous DMF (15 mL) cooled to 0°C was added successively the corresponding alaninamide (1.5 mmol, 0.131 g), HATU (1.5 mmol, 0.570 g), HOAT (1.4 mmol, 0.191 g) and  $KHCO_3$  (15 mmol, 1.5 g). The mixture was stirred during 16 h while allowing it to reach r.t. slowly. The solvent was evaporated at low pressure, the residue was redissolved in  $CH_2Cl_2$  and it was washed with 1M HCl (3 x 10 mL) and sat  $NaHCO_3$  (3 x 10 mL). After evaporation of the organic solvent, the crude product was purified by

flash column chromatography (hexanes/EtOAc, 5:1 or CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 15:1).

**2-(2-((3*R*)-Benzyl-3-[2-(*tert*-butoxycarbonylamino)-(2*R*)-propanamido]-2-oxoazetidin-1-yl)-2-methylpropanamido)-(2*R*)-propanamide (7a):** The general procedure was followed from methyl 2-[[[(3*R*)-3-benzyl-3-[2-(*tert*-butoxycarbonylamino)-(2*R*)-propanamido]-2-oxoazetidin-1-yl]-2-dimethylpropanoate **15** (1 mmol, 0.447 g) and (*R*)-alaninamide **18** (1.5 mmol, 0.131 g). Yield: 0.629 g (80%); white solid; m.p. 190-192°C;  $[\alpha]_D^{25} +36.0$  (0.30, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr)  $\nu_{\max}$  3416, 3308, 2965, 1741, 1726, 1694, 1536, 691 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta_H$  8.3 (s, 1H, NHCCCH<sub>2</sub>Ph), 8.16 (d, 1H, *J* = 6.9 Hz, NHCH(Me)CONH<sub>2</sub>), 7.33-7.28 (m, 5H, Ph), 7.1 (bs, 1H, NH<sub>2</sub>), 6.87 (d, 1H, *J* = 8.1 Hz, BocNH), 6.83 (bs, 1H, NH<sub>2</sub>), 4.13 (m, 1H, BocHNCH(Me), 4.06 (m, 1H, CH(Me)CONH<sub>2</sub>), 3.58 (d, 1H, *J* = 5.1 Hz, CH<sub>2</sub>NCMe<sub>2</sub>), 3.26 (d, 1H, *J* = 5.0 Hz, CH<sub>2</sub>NCMe<sub>2</sub>), 3.05 (d, 1H, *J* = 13.4 Hz, CH<sub>2</sub>Ph), 3.02 (d, 1H, *J* = 13.5 Hz, CH<sub>2</sub>Ph), 1.39 (s, 9H, *t*BuO), 1.29 (d, 3H, *J* = 7.2 Hz, CH(Me)CONH<sub>2</sub>), 1.20 (d, 3H, *J* = 6.9 Hz, BocHNCH(Me), 1.12 (s, 3H, Me<sub>2</sub>CCO), 0.7 (s, 3H, Me<sub>2</sub>CCO). <sup>13</sup>C-NMR (75MHz, CDCl<sub>3</sub>): 175.0, 173.6, 173.2, 166.8, 156.1, 133.1, 130.3, 128.7, 127.8, 80.9, 77.2, 65.8, 59.8, 49.6, 38.9, 28.3, 24.3, 23.6, 15.9, 16.5. HPLC-MS, MeOH/ HCO<sub>2</sub>H *m/z* (ESI, positive polarity): 217.2 (19), 167.1 (33), 149.0 (100), 119.1 (26), 85.9 (29). Anal. Calcd. for C<sub>25</sub>H<sub>37</sub>N<sub>5</sub>O<sub>6</sub>: C, 59.63; H, 7.41; N, 13.91. Found: C, 59.70; H, 7.01; N, 13.91. HRMS (TOF CI) *m/z*: 503.2763; requires: 503.2744.

**2-(2-((3*R*)-Benzyl-3-[2-(*tert*-butoxycarbonylamino)-(2*R*)-propanamido]-2-oxoazetidin-1-yl)-2-methylpropanamido)-(2*S*)-propanamide (7b):** The general procedure was followed from methyl 2-[[[(3*R*)-3-benzyl-3-[2-(*tert*-butoxycarbonylamino)-(2*R*)-propanamido]-2-oxoazetidin-1-yl]-2-dimethylpropanoate **15** (1 mmol, 0.447 g) and (*S*)-alaninamide **18** (1.5 mmol, 0.131 g). Yield: 0.327 g (65%); white solid; m.p. 202-204°C;  $[\alpha]_D^{25} -80.0$  (0.50, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr)  $\nu_{\max}$  3412, 3213, 1733, 1730, 1681, 1629, 696, 734 cm<sup>-1</sup>; <sup>1</sup>H-NMR (500MHz, DMSO-*d*<sub>6</sub>): 8.41 (s, 1H, NHCCCH<sub>2</sub>Ph), 8.30 (d, 1H, *J* = 6.7 Hz, NHCH(Me)CONH<sub>2</sub>), 7.33-7.28 (m, 5H, Ph), 7.10 (s, 1H, NH<sub>2</sub>), 6.88 (d, 1H, *J* = 7.8 Hz, NHBoc), 6.84 (s, 1H, NH<sub>2</sub>), 4.13 (m, 1H, *t*BuOCONHCH(Me), 4.06 (m, 1H, CH(Me)CONH<sub>2</sub>), 3.58 (d, 1H, *J* = 5.3 Hz, CH<sub>2</sub>NCMe<sub>2</sub>), 3.26 (d, 1H, *J* = 5.0 Hz, CH<sub>2</sub>NCMe<sub>2</sub>), 3.06 (d, 1H, *J* = 13.4 Hz, CH<sub>2</sub>Ph),

3.02 (d, 1H,  $J = 13.6$  Hz, CH<sub>2</sub>Ph), 1.39 (s, 9H, *t*BuO), 1.29 (d, 3H,  $J = 7.1$  Hz, CH(Me)CONH<sub>2</sub>), 1.20 (d, 3H,  $J = 6.9$  Hz, BocHNCH(Me), 1.09 (s, 3H, Me<sub>2</sub>CCO), 0.75 (s, 3H, Me<sub>2</sub>CCO). <sup>13</sup>C-NMR (75MHz, CDCl<sub>3</sub>): 175.2, 175.1, 173.5, 173.3, 167.0, 156.1, 133.1, 130.3, 128.7, 127.8, 80.8, 77.2, 65.8, 59.8, 49.7, 47.5, 38.8, 28.3, 24.3, 16.6. HPLC-MS, MeOH/HCO<sub>2</sub>H *m/z* (ESI, positive polarity): 119.1(88), 97.1(60), 91.1(72), 71.1(67), 69.1(63), 57.1(100), 55.1(90). HRMS (TOF CI) *m/z*: 503.2727; C<sub>25</sub>H<sub>37</sub>N<sub>5</sub>O<sub>6</sub> requires: 503.2744.

**2-(2-((3*R*)-Benzyl-3-[2-(*tert*-butoxycarbonylamino)-(2*S*)-propanamido]-2-oxoazetidin-1-yl)-2-methylpropanamido)-(2*S*)-propanamide (7c):** The general procedure was followed from methyl 2-[[[(3*R*)-3-benzyl-3-[2-(*tert*-butoxycarbonylamino)-(2*S*)-propanamido]-2-oxoazetidin-1-yl]-2-dimethylpropanoate **16** (1 mmol, 0.447 g) and (*S*)-alaninamide **18** (1.5 mmol, 0.131 g). Yield: 0.326 g (73%); yellowish solid; m.p. 160-162°C; [ $\alpha$ ]<sub>D</sub><sup>25</sup> +66.0 (0.20, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr)  $\nu_{\max}$  3334, 2969, 1748, 1682, 1536, 1165 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta_{\text{H}}$  8.32 (s, 1H, NHCCCH<sub>2</sub>Ph), 8.16 (d, 1H,  $J = 6.5$  Hz, NHCH(Me)CONH<sub>2</sub>), 7.33-7.28 (m, 5H, Ph), 7.06 (bs, 1H, NH<sub>2</sub>), 6.91 (d, 1H,  $J = 7.0$  Hz, NHBoc), 6.77 (bs, 1H, NH<sub>2</sub>), 4.08 (m, 1H, BocNHCHMe), 4.02 (m, 1H, CH(Me)CONH<sub>2</sub>), 3.61 (d, 1H,  $J = 4.7$  Hz, CH<sub>2</sub>NCMe<sub>2</sub>), 3.24 (d, 1H,  $J = 4.8$  Hz, CH<sub>2</sub>NCMe<sub>2</sub>), 3.05 (d, 1H,  $J = 13.4$  Hz, CH<sub>2</sub>Ph), 3.01 (d, 1H,  $J = 13.5$  Hz, CH<sub>2</sub>Ph), 1.38 (s, 9H, *t*BuO), 1.31 (d, 3H,  $J = 7.2$  Hz, CH(Me)CONH<sub>2</sub>), 1.20 (d, 3H,  $J = 7.2$  Hz, BocNHCHMe), 1.12 (s, 3H, Me<sub>2</sub>CCO), 0.7 (s, 3H, Me<sub>2</sub>CCO). <sup>13</sup>C-NMR (75MHz, CDCl<sub>3</sub>): 175.0, 173.6, 173.0, 166.7, 155.9, 133.0, 130.3, 128.7, 127.8, 80.9, 75.8, 65.9, 59.8, 49.6, 47.6, 38.9, 28.3, 24.3, 23.6, 16.5. HPLC-MS, MeOH/HCO<sub>2</sub>H *m/z* (ESI, positive polarity): 187.1(49), 133.1(37), 91.1(100), 70.1(60), 57.1(42). Anal. Calcd. for C<sub>25</sub>H<sub>37</sub>N<sub>5</sub>O<sub>6</sub>: C, 59.63; H, 7.41; N, 13.91. Found: C, 59.85; H, 7.34; N, 13.92. HRMS (TOF CI) *m/z*: 503.2771; requires: 503.2744.

**2-(2-((3*R*)-Benzyl-3-[2-(*tert*-butoxycarbonylamino)-(2*S*)-propanamido]-2-oxoazetidin-1-yl)-2-methylpropanamido)-(2*R*)-propanamide (7d):** The general procedure was followed from methyl 2-[[[(3*R*)-3-benzyl-3-[2-(*tert*-butoxycarbonylamino)-(2*S*)-propanamido]-2-oxoazetidin-1-yl]-2-dimethylpropanoate **16** (1 mmol, 0.447 g) and (*R*)-alaninamide **18** (1.5 mmol, 0.131 g). Yield: 0.392 g

(77%); yellowish solid; m.p. 219-220°C;  $[\alpha]_D^{25} -15.9$  (0.20, CH<sub>2</sub>Cl<sub>2</sub>); IR (KBr)  $\nu_{\max}$  3413, 3303, 2978, 2914, 1744, 1663, 1542 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>)  $\delta_H$  8.53 (s, 1H, NHCCH<sub>2</sub>Ph), 8.37 (d, 1H, *J* = 7.3 Hz, NHCH(Me)CONH<sub>2</sub>), 7.33-7.28 (m, 5H, Ar), 7.03 (d, 1H, *J* = 6.7 Hz, BocNH), 6.97 (s, 1H, NH<sub>2</sub>), 6.88 (s, 1H, NH<sub>2</sub>), 4.04 (m, 1H, BocNHCHMe), 4.03 (m, 1H, CH(Me)CONH<sub>2</sub>), 3.46 (d, 1H, *J* = 4.8 Hz, CH<sub>2</sub>NCMe<sub>2</sub>), 3.34 (d, 1H, *J* = 5.0 Hz, CH<sub>2</sub>NCMe<sub>2</sub>), 3.06 (d, 1H, *J* = 13.4 Hz, CH<sub>2</sub>Ph), 3.01 (d, 1H, *J* = 13.4 Hz, CH<sub>2</sub>Ph), 1.38 (s, 9H, *t*BuO), 1.31 (d, 3H, *J* = 7.2 Hz, CH(Me)CONH<sub>2</sub>), 1.19 (d, 3H, *J* = 7.0 Hz, BocHNCHMe), 0.97 (s, 3H, Me<sub>2</sub>CCO), 0.79 (s, 3H, Me<sub>2</sub>CCO). <sup>13</sup>C-NMR (75MHz, CDCl<sub>3</sub>): 175.9, 173.4, 172.5, 167.7, 155.9, 132.7, 130.3, 128.8, 127.9, 80.8, 77.2, 65.6, 58.9, 49.5, 48.1, 38.8, 28.3, 23.7, 17.3. HPLC-MS, MeOH/HCO<sub>2</sub>H *m/z* (ESI, positive polarity): 187.1 (44), 173.1 (28), 172.1 (26), 117.1 (37), 116 (25), 115 (26), 113.1 (35), 91 (100), 70.0 (47). Anal. Calcd. for C<sub>25</sub>H<sub>37</sub>N<sub>5</sub>O<sub>6</sub>: C, 59.63; H, 7.41; N, 13.91. Found: C, 59.21; H, 7.60; N, 13.79. HRMS (TOF CI) *m/z*: 503.2742; requires: 503.2744.

**Acknowledgement.** We thank the Ministerio de Ciencia y Competitividad (MEC, Spain) (Project: CTQ2010-21625-C02-01), Gobierno Vasco (ETORTEK-nanoIKER IE-11/304) and Universidad del País Vasco UPV/EHU (UFI QOSYC 11/22) for financial support, and SGIker UPV/EHU for NMR facilities. Grants from Gobierno Vasco to M. S.-A. and from UPV/EHU to E. A. are acknowledged.

**Supporting Information Available.** <sup>1</sup>H NMR spectra of compounds **5-7** and **13d-e**. <sup>13</sup>C NMR spectra of compounds **5-13b-c**, **15** and **16**. NMR/ROESY spectra and inter-proton distances of compounds **5-7**. Gaussian output data of structures **5a-f**. CIF files for **5e**, **5f**, **7a** and **7d**. This material is available free of charge via the Internet at <http://pubs.acs.org>.



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